INVESTIGATING THE THERAPEUTIC EFFECTS OF SILVER AND GOLD NANOPARTICLES CAPPED WITH SELECTED MEDICINAL PLANTS AGAINST TUBERCULOSIS.

BY

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17001844

A DISSERTATION SUBMITTED IN FULFILMENT FOR THE REQUIREMENT OF MASTER'S DEGREE IN MICROBIOLOGY

TO THE

DEPARTMENT OF BIOCHEMISTRY & MICROBIOLOGY

IN THE

FACULTY OF SCIENCE, ENGINEERING AND AGRICULTURE

UNIVERSITY OF VENDA

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FEBRUARY 2023

ABSTRACT

Background: Tuberculosis (TB) is implausibly still considered as one of the leading causes of death, in the 21st century. Despite the curative treatments and measures of control in the communities, TB plays a significant role in human infectious disease. Studies have shown that there is an emergence of major drug-resistant TB.

Aim: This study was aimed at evaluating the biological activities of silver and gold nanoparticles capped with selected medicinal plant extracts against *Mycobacterium tuberculosis.*

Methodology: *P. africanum* and *Z. mucronata* barks and leaves were collected in the Vhembe district of Limpopo. Plant phytochemical constituents were extracted using distilled water and absolute methanol. The silver nanoparticles (AgNP) and gold nanoparticles (AuNP) were capped using crude extracts through the process of green synthesis; and were characterized by Ultraviolet-Visible spectrophotometry (UV-VIS), and transmission electron microscopy (TEM). Metabolites present in the plant extracts were profiled using liquid chromatography mass spectrometry (LC-MS). Cytotoxicity activity of plant extracts and nanoparticles were determined by MTS assay on HEK293 cells. The anti-inflammatory activity was determined through the nitric acid synthase (NOS) inhibitory test. The antimycobacterial activity was determined using microbroth dilution.

Results: Following extraction by maceration, methanol was able to yield more extracts compared to distilled water due to their differences in polarity. The selected plants were found to contain numerous antioxidant significant for anti-inflammatory and cytotoxic activity. The metabolites play a role in the formation of the nanoparticles. Plant extracts and gold nanoparticles had little impact on the cell lines, thus were concluded to be non-toxic. Whilst silver nanoparticles exhibited toxic activity on the cell line at both concentrations, hence were considered toxic to human. Silver nanoparticles of ZML and ZHL, and gold nanoparticles and plant extracts of PML exhibited anti-inflammatory activity at 100 µg/mL, whereas PML was able to decrease nitrite concentration at both concentrations.

Conclusion: Adoption of TB strategies recommended by World Health Organization (WHO) to reduce TB deaths and incidence rate by 90% and 80%, respectively, (less than 20 TB cases per 100 000 population).

Keywords: DR-TB, Nanoparticles, *Peltophorum africanum,* Tuberculosis, *Ziziphus mucronate*

DECLARATION

I, Matshoene Violet Motene, declare that this dissertation is my original work and has not been submitted for any degree at any other institution or University. The dissertation does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signed:  Date: 16 May 2023

LIST OF ABBREVIATIONS

µl	Microliter
G	Grams
Ml	Milliliters
mg/ml	Milligrams per milliliter
Nm	Nanometer
Min	Minutes
Mm	Millimeters
mM	Millimolar
AgNO3	Silver nitrate
CO2	Carbon dioxide
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulpfophenyl)-2H-tetrazolium)
TEM	Transmission electron microscopy
XRD	X-ray diffraction
UV-VIS Spec	Ultraviolet visible spectrophotometry
UV	Ultraviolet
M. tuberculosis	*Mycobacterium tuberculosis*
Z. mucronate	*Ziziphus mucronata*
P. africanum	*Peltophorum africanum*
TB	Tuberculosis
DR-TB	Drug resistant tuberculosis
HIV	Human immunodeficiency virus
MtbC	Mycobacterium tuberculosis complex
LCMS	Liquid chromatography mass spectroscopy
STI	Sexual transmitted infections
AuNP or (g)	Gold nanoparticles
AgNP or (s)	Silver nanoparticles

µg/ml	Microgram per mililiters
IC50	Half maximal inhibitory concentration
PHL	Water extracts of *Peltophorum africanum* leaves
PHB	Water extracts of *Peltophorum africanum* bark
PML	Methanol extracts of *Peltophorum africanum* leaves
PMB	Methanol extracts of *Peltophorum africanum* bark
ZHL	Water extracts of *Ziziphus mucronata* leaves
ZHB	Water extracts of *Ziziphus mucronata* bark
ZML	Methanol extracts of *Ziziphus mucronata* leaves
ZMB	Methanol extracts of *Ziziphus mucronata* bark
NO	Nitric oxide

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Tuberculosis (TB) is an ancient disease with origins that dates back to 500 BCE (Gerszten et al., 2001). Tuberculosis is still considered as one of the leading causes of death in the 21st century (Camirero et al., 2021). Despite the curative treatments and measures of control in the communities, TB plays a significant role in human infectious disease. The World Health Organization (2020) reported that in 2018, there were 10 million new cases of tuberculosis worldwide with mortality rate ranging up to 1.5 million.

In most patients with human immunodeficiency virus (HIV), the leading cause of death is tuberculosis (WHO, 2015; WHO, 2011a). Patients that are HIV positive become susceptible to TB, often get infected with this disease prior symptoms of acquired immunodeficiency syndrome (AIDS) become apparent (WHO, 2020a; WHO, 2008b; Lall & Meyer, 2001). World Health Organization (2018) has shown that there is an emergence of major drug-resistant TB.

One of the key factors leading to the growth in global tuberculosis incidence since 1980 is the multi drug-resistant *Mycobacterium tuberculosis* strains (WHO, 2015). Multidrug resistant (MDR) tuberculosis variants are characterized as *M. tuberculosis* strains that are resistant to at least Isoniazid and Rifampicin, which are the first-line therapies used in tuberculosis treatment (WHO, 2011b; Lawn & Wilkison, 2006). Furthermore, tuberculosis caused by resistant strains to first-line therapies, fluoroquinolones, plus at least one of three injectable second-line treatments, such as capreomycin, kanamycin, and amikacin, is known as Extensive Drug Resistant (XDR) TB (WHO, 2011b; Lawn & Wilkison, 2006).

TB is a major global concern since it infects millions of people each year (Madisha et al., 2016), governing the critical need of new anti-TB drugs. The evolution of antimicrobial resistance in TB has become a major public health concern in many developing countries. Prolonged treatment and affordability of anti-TB drugs are the major factors affecting treatment outcome. As a result, there is an immediate need for new, low-cost TB medications with high efficacy and fewer side effects.

Plants have been shown to be a valuable source of future pharmaceuticals and a good option for drug prospecting in the management and treatment of infectious diseases (Eddouks et al., 2012; Street & Prinsloo, 2012). African culture is replete with medicinal plants (Buwa & Afolayan, 2009) and antimicrobial agents are known to be produced by many higher plants (van Wyk & Gericke, 2003). Medicinal plants all around the globe have been discovered to have antibacterial effects (Street & Prinsloo, 2012; Mann et al., 2008). However, local herbalists who use plants for medicinal purposes have no scientific knowledge of the structural functions of the chemicals in the herbs before prescribing to patients. Therefore, laboratory screening of these herbs is required to validate the medicinal uses of plants and to assess the toxicity level and components of such plants.

Nanoparticles are the breakthrough to medicinal studies. Application of nanoparticles allows for early detection of diseases and decreases damage to healthy cells in the body because they are developed to be attracted to sickly cells, permitting direct treatment directly on these cells. In a study conducted by Kasithevar et al. (2017), it was observed that nanoparticles affect the permeability of cell membrane and deteriorate cell growth behavior, thus restricting replication of the pathogenic cells and eventually resulting in cell death (Ponsanti et al., 2020). Nanotechnology has gained a lot of interest and has a wide range of processes that decrease toxic substances to remediate the environment. Modern alternative metal nanoparticle synthesis includes the use of inactivated plant tissue, plant extracts, exudates, and other parts of living plants (Parson et al., 2007). Currently, nanoparticles are developed for drug, heat, and light delivery to specific cells (Huong and Thang, 2020).

1.2 PROBLEM STATEMENT

Studies have shown that there is a rapidly aggressive emergence of multi-drug resistant *Mycobacterium tuberculosis* strain that contributes to global health problems, as represented by the current tuberculosis status and antimycobacterial-drug crisis (Tăbăran et al., 2020). This is influenced by lack of TB status monitoring in rural areas, linked with increasing incidences of HIV and AIDS. World Health Organization reported that in 2018, there were 10 million new cases of tuberculosis worldwide with mortality rate ranging up to 1.5 million (WHO, 2019).

South Africa has been reported to have the eighth highest TB burden in the world; with nearly 360 000 people infected with TB in year 2019, of which 58 000 died from the disease, according to National Institute of Communicable Diseases (NICD). In 2021, TB was the leading cause of death. This shows that there is an urgent need of development of anti-TB drugs for treatment of emerging TB diseases in South Africa. With the discovery of potential anti-TB medicinal plants and nanoparticles may provide solutions as alternative anti-TB treatment therapy.

1.3 STUDY RATIONALE

Recent investigations have revealed an upsurge in tuberculosis infections. *Mycobacterium tuberculosis* has developed resistance to medications such as rifampicin and isoniazid, which were once considered the greatest treatment of this disease (Caminero et al., 2021; WHO, 2019). According to the WHO (2018), there were approximately 490 000 incidents of rifampicin-resistant tuberculosis (RR-TB) worldwide, with isoniazid-resistant tuberculosis accounting for 78% of those cases.

It was reported that when patients with tuberculosis are treated effectively, they become non-infectious quickly, but untreated or undetected TB cases cause the most transmission (Nardell and Dharmadhikari 2010; Santha et al., 2002). Therapy failure, relapse, poor adherence to past treatment by the patient, and treatment after default are just some of the issues that make tuberculosis management difficult (Caminero, 2005). Other variables include HIV infection and diabetes, as well as the long-term use of anti-TB drugs (Jibrin et al., 2012).

Nanotechnology and nanoparticle research has yielded a deal of fascinating new tactics and ideas that could help to improve TB treatment. The model of new anti-tuberculosis therapy based on gold and silver nanoparticles has the potential to curb the resistance of TB to treatments (Tăbăran et al., 2020).

This current study focused on providing potential solutions for prevention of emergence and transmission of drug-resistant tuberculosis (DR-TB) as the second line of treatment has less efficacy against the disease, requires long treatment duration and have toxic side effects (Zager and Nerney, 2008; WHO, 2007). Furthermore, failure of the currently available treatment subsequently led to relapse and high mortality rates (WHO, 2019).

To counter the challenges associated with tuberculosis resistance and treatment failure, finding, or producing therapeutic drugs that are administered in short and single courses may be of solution. Thus, this study will investigate whether silver and gold nanoparticles capped with selected medicinal plant materials provide a short and effective treatment.

1.3 OBJECTIVES

1.3.1 PRIMARY OBJECTIVE

The primary objective of this study was to evaluate the biological activities of selected medicinal plant capped silver and gold nanoparticles against *Mycobacterium tuberculosis*.

1.3.2 SECONDARY OBJECTIVES

The secondary objectives of this study were to:

- Profile compounds in the crude extracts using LC-MS
- Nanoparticle synthesis using plants by green synthesis technology
- Characterize the synthesized silver and gold nanoparticles using transmission electron microscope (TEM)
- Determine the toxicity using the MTS assay and anti-inflammatory activity using the nitric oxidase assay
- Determine antimycobacterial activity of the crude extracts and nanoparticles using broth-microdilution assay

1.4 RESEARCH QUESTIONS

- Do metal nanoparticles have different biological activities based on their nature?
- Will the nanoparticles have enhanced biological activities compared to the medicinal plants?

- Which metal nanoparticles will provide the solution to emergence of drug resistant TB?

CHAPTER 2

LITERATURE REVIEW

2.1 TUBERCULOSIS

2.1.1 INTRODUCTION

Tuberculosis (TB) is a disease that is caused by bacilli bacteria called *Mycobacterium tuberculosis*, in humans (Kaforou et al., 2022). The disease is transmitted through inhalation of aerosolized droplets infected with *M. tuberculosis*; and affects the lungs and extrapulmonary sites of the human body (WHO,2013). TB is primarily caused by *Mycobacterium tuberculosis*, and to a lesser extent, infections with other mycobacteria such as *M. africanum, M. bovis, M. canetti, M. caprae, ,* and on rare occasions*, M. pinnipedii or M. microti* (Thoen et al., 2014; Kiers et al., 2008; Richter et al., 2003). The exact involvement of several newly integrated members of *MtbC*, such as *M. mungi*, in human tuberculosis is yet unknown (Alexander et al., 2010). *MtbC* bacteria are non-motile bacilli with a thick, non-sporulated, lipid-rich cell wall that belong to the *Actinomycetales* order (Tăbăran et al., 2020). The advent of drug resistant *MtbC* strains in recent decades has posed new obstacles to anti-TB control and prevention efforts.

2.1.2 PATHOGENESIS AND VIRULENCE FACTORS OF MYCOBACTERIUM TUBERCULOSIS

The pathogenicity of *MtbC* is established by the abundance of virulence factors and literature dedicated to these factors is vast (Echeverria et al., 2018; Smith, 2003). This plays a crucial development of the disease, giving tuberculosis a peculiar course of biological events and manner of interacting with immune cells (Tăbăran et al., 2020). The virulence factors of *MtbC* were classified into nine categories by Forrellad et al. (2013), according to their bacterial location, chemical structure and their activity: (i) bacterial-wall proteins and lipoproteins (including secretion systems cell wall), (ii) proteases, (iii) regulator gene, (iv) virulence factors involved in the metabolism of lipids

and fatty acids (v) protein kinases, (vi) proteins involved in metal transport, (vii) proteins that suppress the antimicrobial effectors of macrophage, (viii) proteins of unknown function and (ix) other virulence proteins.

2.2 NANOTECHNOLOGY AND NANOPARTICLES

2.2.1 INTRODUCTION

Nanoparticle science and nanotechnology have provided innovative and practical solutions for diseases caused by bacterial infections and several critical issues by connecting interdisciplinary areas of research such as medicine, physics, and chemistry (Sathiyavimal et al., 2018). Although metallic silver has a long history of use in medical, its popularity distinctly declined following the development and broad usage of antibiotics (Shankar et al., 2016).

Nanoparticles' main advantage is their usefulness in transportation and delivery of wide array of molecules; and have a high loading capacity of substances (Kesharwani, 2020). Nanoparticles have different biological and structural properties; these properties play roles in the increase of solubility, protection of the content and capsule, bioavailability and uptake of the molecules transported (Kesharwani 2020).

Recent reports have shown that nanoparticles have a high antimycobacterial effect in both bacterial cultures and within macrophages, thus, the exploration of this new-concept of antimycobacterial-nanoparticles could change the current optics regarding TB-therapy (Montelongo et al., 2019; Mohanty et al., 2013).

2.2.2 NANOPARTICLES AND TUBERCULOSIS

With the increased interest in nanoparticle field, nanoparticles can be used for drug delivery, detection of infections, and enhancement of anti-TB drugs (Hussain et al., 2013; Anisimova et al., 2000). In a study performed by Zahoor et al. (2005), testing inhalable alginate nanoparticles' efficacy of drug delivery in guinea pigs for treatment of tuberculosis. The guinea pigs were nebulized with antitubercular drugs that were gelificated with alginate nanoparticles. It was found that 15 days post nebulization, the drugs were still detectable in the spleen, lungs, and liver; whilst nanoparticle free drugs stayed in animals' system for 1 day. The nanoparticles controlled or delayed the removal time of the antituberculosis drugs from the animals' system, thus, they can

serve as ideal carriers of controlled release of anti-TB drugs. A similar study by Anisimova et al. (2000), found that the synergy of nanoparticles and anti-TB drugs (Isoniazid and Streptomycin) enhanced antimicrobial effects on the *M. tuberculosis*.

2.2.3 SYNTHESIS OF NANOPARTICLES

A. SILVER NANOPARTICLES

Green synthesis of silver nanoparticles has gained significant interest over chemical and physical procedures (Shedbalkar et al., 2014; Thakkar et al., 2010). Formation of silver nanoparticles is dependent on reduction of Ag^+ by a combination of biomolecules such as amino acids, enzymes, polysaccharides, proteins, and vitamins (Anupam et al., 2019). As shown in Figure 2.1, the biomolecules reduce nitrate (NO_3^-) to nitrite (NO_2^-); in this reduction process, an electron is donated to a silver ion (Ag^+) which is then reduced to a metallic silver (Ag^0) (Kalimuthu et al., 2008).

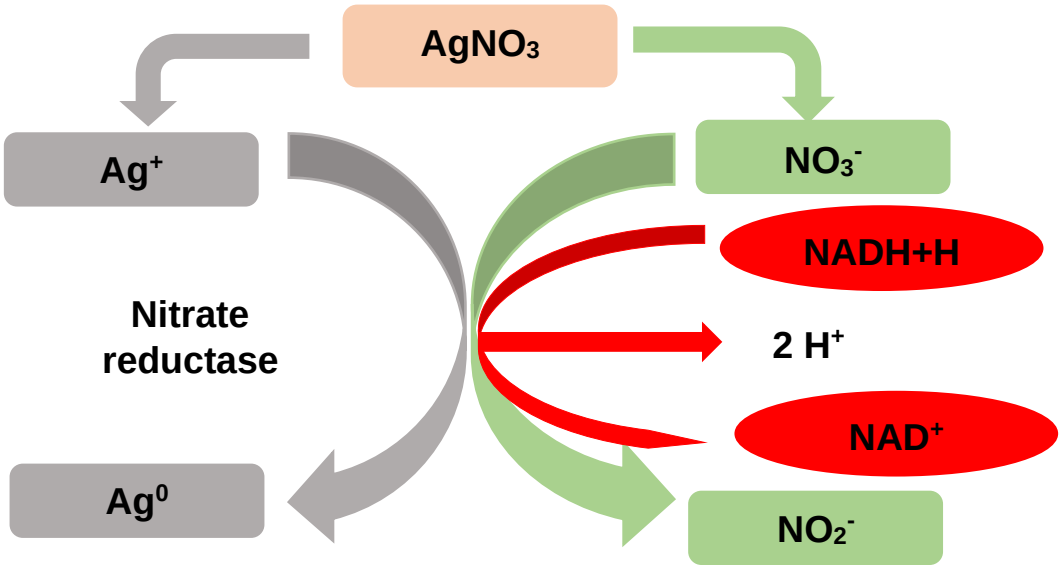


Figure 2.1: Mechanism of synthesis of silver nanoparticles mediated by nitrate synthase enzyme.

B. GOLD NANOPARTICLES

Gold nanoparticles are a promising solution to a variety of biomedical challenges (Mikhailova, 2021). The synthesis and formation of gold nanoparticles is dependent on the biomolecules (such as flavonoids and phenolic acids) present in the plant extracts; the biomolecules form an intermediate complex with Au^{3+}, which undergoes oxidation to keto-forms, resulting in reduction of the trivalent gold into gold

nanoparticles (El-Borady et al., 2020; Islam et al., 2019; Vijaya et al., 2019; Rajan et al., 2015).

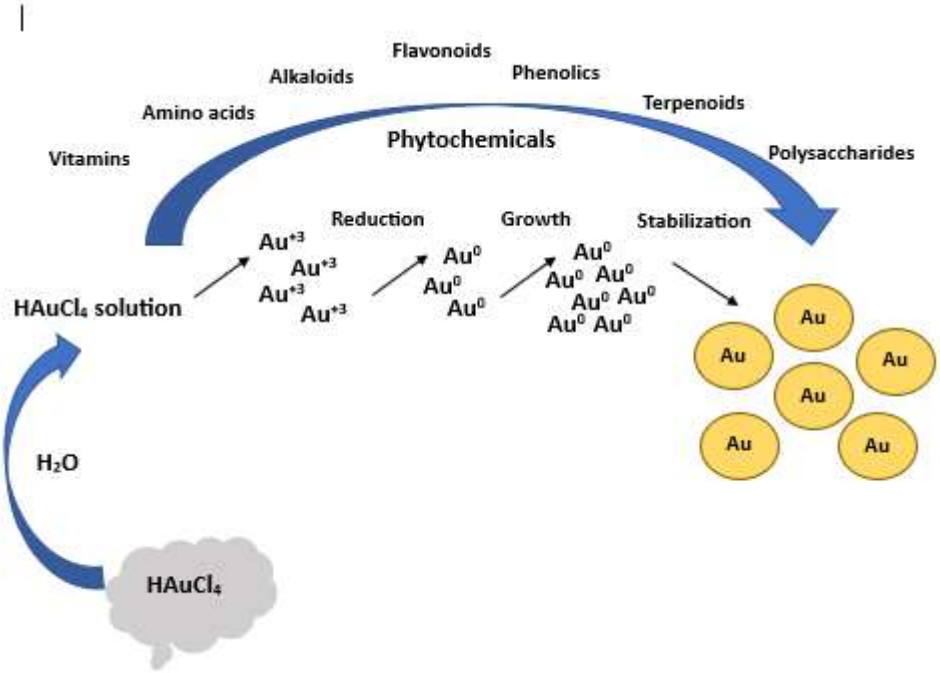


Figure 2.2: Mechanism of plant extract mediated AuNP synthesis and stabilization. Image adapted from https://www.researchgate.net/figure/General-mechanism-of-plant-extract-mediated-synthesis-and-stabilization-of-AuNPs_fig1_315797627.

2.3 BACKGROUND ON MEDICINAL PLANTS

2.3.1 INTRODUCTION

Medicinal plants have been used since the beginning of human civilization. Numerous cultures have found remedies within their habitat and are still relying on indigenous medicinal plants for health care needs (Eddouks et al., 2012; Street and Prinsloo, 2012). South Africa accounts for almost 10% of the world's higher plant species, with a variety of around 30,000 flowering plant species (van Wyk and Gericke, 2003; Louw et al., 2002). These plants are widely used for several human health, social, and economic support systems. Furthermore, benefit studies have revealed the variety and complexity of compounds that contribute to various usages of plants in treating numerous ailments including life-threatening diseases such as diabetes, cancer, and HIV/AIDS (Street and Prinsloo, 2012).

2.3.1 PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANTS OF MEDICINAL PLANTS

Medicinal plants contain vast range of phytochemicals. Bioactive phytochemicals or secondary metabolites have been reported to have biological activities such as immune system activation, antimicrobial influence, anti-cancer, detoxification of enzyme modulation, antioxidant activity, anti-inflammatory, anti-diabetic, and cardiovascular disease (Leonov et al., 2015; Chandra et al., 2014; Sasidhana et al., 2011). Though the phytochemicals are not nutritionally necessary, they play a role in prevention of diseases.

The secondary metabolites found in plants are varied, allowing production of several drug extracts that can be tested on host cells to assess toxicity levels before usage as alternative treatment for diseases (Rauf et al., 2013). Classes of phytochemicals such as saponins, flavonoids, alkaloids, phenols, and terpenoids are among the bioactive compounds; the combination of these compounds may be responsible for the efficacy of the plant extracts. Mazid et al. 2011, reported that therapeutic phytochemical compounds are obtainable in all parts of the plants, that is, roots, barks, seeds, and bulbs.

The phytochemical compounds with antioxidant activity play a major role in reduction of oxidant and reactive oxygen species that cause diseases and human disorders (Halliwell et al., 1981). The antioxidant agents function by deactivating or stabilizing free radicals to prevent them from attacking healthy cells within humans (Djeridane et al., 2006). Medicinal plant extracts with abundant antioxidant agents have been found to have potential therapeutic activity against diseases (Govind, 2011).

2.4 PLANTS USED IN THIS STUDY

2.4.1 *ZIZIPHUS MUCRONATA (WILLD.)*

Ziziphus mucronate (Willd.) commonly known as Buffalo thorn (English), Mutshetshethe (Venda), Mokgalo, Moonaona (Sotho), is a medium sized tree belonging to *Rhamnaceae* family (Magwede et al., 2018).

Figure 2.3: An image of *Ziziphus mucronata* leaves and fruits. *Z. mucronata* is a hardy, medium sized, deciduous tree that has both straight and hook thorns. The leaves are glossy that turn golden yellow in autumn seasons. Image adapted from https://www.plantbook.co.za/ziziphus-mucronata/ on 17 November 2022.

Ziziphus mucronata is abundant in areas that are dominated by thorny vegetation in both tropical and temperate climates. It is widely spread in South Africa, Zimbabwe, Senegal, and Chad (Maier et al., 2006). This plant is used to treat various STIs in some regions of Zambia (Chinsembu, 2016); and is used to remedy glandular swellings, measles, tuberculosis and boils in South Africa (Olajuyigbe and Afolayan, 2012). The dried roots or bark is crushed and boiled in water; one cup of the decoction is drunk three times daily (Chinsembu et al., 2019).

2.4.2 *PELTOPHORUM AFRICANUM (SOND.)*

Peltophorum africanum (Sond.) commonly known as African wattle (English), Musese (Venda), Mosehla, Motlepe, Mosese (Sepedi), belonging to *Fabaceae* family.

Figure 2.4: Image of *Peltophorum africanum*. The tree is medium sized with spreading and sparse crown and is frequently branched from near the bottom of the bark. The bark is brown in color, fissured and rough with crevices. Image adapted from https://www.plantbook.co.za/peltophorum-africanum/ on 17 November 2022.

This plant is widely spread across in the northern provinces of South Africa, namely Limpopo, Gauteng, Mpumalanga, KwaZulu-Natal and North West (Reynolds, 2001). *Peltophorum africanum* is used to treat tuberculosis and chronic cough, HIV and gonorrhea (Chinsembu et al., 2019; Semenya and Maroyi, 2019). The dried bark is boiled for 5 to 11 minutes, and the extracts are taken orally, three times per day (Semenya and Maroyi, 2019).

2.5 SUMMARY OF THE LITERATURE REVIEW

Tuberculosis is a disease caused by Mycobacterium tuberculosis. and is one of the leading killer diseases globally. The disease is spread through inhalation of infected air droplets and affects the lungs and extrapulmonary parts of the human body. The spread and recurrence of tuberculosis is influenced by the emergence of resistant strains of M. tuberculosis, and current anti-TB treatments are becoming obsolete. This encourages new production of anti-TB therapy methods.

Some medicinal plants contain secondary metabolic compounds that have been reported to have anti-tuberculosis properties. Secondary metabolites have been reported to have biological activities such as immune system activation, antimicrobial influence, anti-cancer, detoxification of enzyme modulation, antioxidant activity, anti-inflammatory, anti-diabetic, and cardiovascular disease (Leonov et al., 2015; Chandra et al., 2014; Sasidhana et al., 2011). Though the phytochemicals are not nutritionally necessary, and sustain life of humans, they play a role in prevention of diseases.

The secondary metabolites found in plants are varied, allowing production of several drug extracts that can be tested on host cells to assess toxicity levels before usage as alternative treatment for diseases (Rauf et al., 2013).

Nanoparticles have shown great importance in transportation and delivery of wide array of molecules; and have a high loading capacity of substances (Kesharwani, 2020). Nanoparticles have different biological and structural properties; these properties play roles in the increase of solubility, protection of the content and capsule, bioavailability and uptake of the molecules transported (Kesharwani 2020).

Thus, with the properties of nanoparticles and the compounds found in plants, the synergy may be of solution for treatment and prevention of re-emergence of resistant M. tuberculosis strains.

CHAPTER 3

MATERIALS AND METHODS

3.1 ETHICAL CLEARANCE

This study is part of a much broader research project, ethical approval was obtained from the Health, Safety and Research Ethics Committee of the University of Venda (SMNS/14/MBY/30/1210). The medicinal plants were purchased from traditional healers.

3.2 CHEMICALS AND REAGENTS

Chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO, USA) such as Dimethyl Sulfoxide (DMSO), Ammonium Hydroxide was purchased from Rochelle chemicals (Gauteng, RSA), Methanol was obtained from Merck chemical (Kenilworth, NJ; USA), Silver nitrate was bought from Associated Chemical Enterprise (Gauteng, RSA).

3.3 PLANT MATERIALS AND STORAGE

Plant leaves and tree barks were collected at Shakadza, Vhembe district, with the aid of traditional herbalists to help identify the plant. The leaves and barks were washed with clean water to remove potential contaminants and soil residues, then cut into small pieces (between 2-4cm in length, 1cm width) before drying at 27°C for 2 weeks. The dried leaves and barks were ground using a Retsch SM 100 grinder (Thermo Fischer Scientific, Waltham, MA, USA) and stored in a clean, empty plastic bag in darkness, at room temperature, until further use.

3.3 PREPARATIONS OF EXTRACTS

The plant compounds were extracted using absolute Methanol and distilled water. An amount of 50 g of the ground tubers were weighed and soaked at room temperature for 72 hours in 500 ml of each solvent in 1000 ml bottles (Das et al., 2010). The supernatants were centrifuged using a Heraeus Multifuge X3R centrifuge (Thermo

Fisher Scientific, Waltham, MA, USA) at 5000 rpm for 10 minutes at 4℃. The extracts were concentrated using a rotary evaporator (BUCHI, Flawil, Switzerland).

3.4 LCMS ANALYSIS

Phytochemical analysis was done following the method performed by Akpalo et al. (2020), with slight modifications; using LC-QTOF-MS, model LC-MS 9030. The compounds were detected between 200 and 600 nm of 4 nm step. Mobile phase of the high-performance liquid chromatography (HPLC) consisted of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile: 0.1% formic acid (1:1, v/v) (solvent B). A 100 mm x 2.1 mn with particle size of 2.7 µm C18 column (Shim Pack Velox, Shimadzu, Kyoto, Japan) was operated at 26°C, with injection volume of 5 µl, and 1 ml/min flow rate. The compounds were identified using liquid chromatography mass spectrometry (LC-MS) based on their absorbance spectrum, retention time and comparation of mass fragmentation from literature.

3.5 SYNTHESIS OF SILVER AND GOLD NANOPARTICLES

Green synthesis of silver and gold nanoparticles was done as described by Tamilarasi and Meena (2020), with slight modifications. Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) and gold tetrachloroaurate ($AuCl_4$) were prepared and used for the synthesis of silver and gold nanoparticles. A 6 ml of plant extracts were added to each 40 ml of 1 mM $AgNO_3$ and $AuCl_4$ solutions. The synthesis of silver and gold nanoparticles were carried out at room temperature for 24 hours in dark conditions. The nanoparticle solution thus obtained were purified by repeated centrifugation at 14,000 rpm for 20 minutes at ambient temperature, followed by redispersion of the pellet of silver and gold nanoparticles into acetone. After air drying of the purified nanoparticles, they were stored in 15 ml Eppendorf tubes; at 4℃ for silver nanoparticles and room temperature for gold nanoparticles, further analysis.

3.6 CHARACTERIZATION OF NANOPARTICLES

3.6.1 TRANSMISSION ELECTRON MICROSCOPY (TEM)

Transmission electron microscopy T(EM) analysis were done to visualize the shape as well as the morphology of the green-synthesized silver and gold nanoparticles. In this study, only nanoparticles that exhibited biological activity were characterized. The nanoparticles were dissolved in 70% ethanol. A drop of the solution was dispersed on a carbon capped copper grid and dried at room temperature. Then screened in JEM2100F Transmission Electron Microscope (JEOL, Musashino, Japan). The sizes of the nanoparticles were measured using the ImageJ software version 1.52 v and size distribution were determined using Origin software.

3.7 CYTOTOXICITY ASSAY

3.7.1 SAMPLE PREPARATION

The nanoparticles and plant extracts were reconstituted in DMSO at a stock concentration of 100 mg/mL and were stored at 4°C for further use.

3.7.2 CELL LINE MAINTENANCE

The Vero cells (African green monkey kidney cell line) were purchased from Cellonex, South Africa. The cells were maintained in DMEM with 10% FBS, in 10 cm culture plates and incubated at 37°C in a humidified atmosphere with 5% CO_2.

3.7.3 MTT ASSAY

Amounts of 100 µl of the cells were seeded in 96-well plates at 4000 cells/well and left overnight to attach. The sample treatments were prepared in complete medium and 100 µl was added to cells and were incubated for 24 hours. The treatments were aspirated, and 100 µl of 0.5 mg/mL MTT in complete medium was added to each well. The cells were incubated for 3 hours. DMSO at 100 µl was added each well and absorbance was measured at 540 nm using a BioTek PowerWave XS spectrophotometer (Winooski, VT, USA).

3.8 ANTI-INFLAMMATORY TESTING

3.8.1 SAMPLE PREPARATION

The nanoparticles and plant extracts were solubilized using DMSO to make a 100 mg/mL stock solution. The samples were stored at 4°C until used. Aminoguanidine (AG) was used as a positive control.

3.8.2 ANTI-INFLAMMATORY SCREENING PROTOCOL

This procedure was performed as described by Mabasa et al. (2021). RAW 264.7 cells were seeded in RPMI1640 culture medium supplemented with 10% FBS into 96-well plates at a density of 1×10^5 cells per well and allowed to attach overnight. After 24 hours, the culture medium was removed and 50 µL sample aliquots were added to give a final concentration of 50 and 100 µg/mL. To assess anti-inflammatory activity, 50 µL of LPS containing medium was added to the corresponding wells. A 100 µL of aminoguanidine was added as a positive control. Cells were incubated for 24 hours.

To quantify the nitric oxide (NO) production, 50 µL of the spent medium was transferred to a new 96-well plate. Sulfanilamide solution and NED solution was prepared as per manufacturer's instructions. Sulfanilamide solution was added to the spent culture medium at 50 µL and incubated for 10 minutes at room temperature in the dark. The NED solution was the added to each well at 50 µL and further incubated for 10 minutes at room temperature in the dark. The absorbance was measured at 540 nm (BioTek PowerWave XS spectrophotometer). A standard curve using sodium nitrate dissolved in the culture medium was used to determine the concentration of NO in each sample.

3.8.3 CYTOTOXICITY SCREENING PROTOCOL

To confirm the absence of toxicity as a contributory factor, cell viability was assessed using MTT. This was done by the removal of the remaining of the medium and treatments in each well and replacing it with medium containing 0.5 mg/mL and incubated for 30 minutes at 37°C. Thereafter the MTT was removed and 100 µL DMSO was added to each well to solubilise the formazan crystals. Absorbance was measure at 250 nm using a BioTek PowerWave Xs spectrophotometer (Winooski, VT, USA).

3.9 ANTIMICROBIAL TESTING

The antimycobacterial activity of the plant extracts, silver and gold nanoparticles was tested against *Mycobacterium smegmatis* mc^{2}155. The *M. smegmatis* was maintained in Middlebrook 7H9 (Fluka M7H9) broth that was supplemented with glycerol, Middlebrook growth supplement OADC (Oleic Albumin Dextrose Catalase), and tween 80 at 37˚C.

The minimum inhibitory concentration (MIC) to inhibit the growth of the *M. smegmatis* was determined following a procedure described by Eloff (1998), with moderate change for Mycobacteria (McGaw et al., 2008). The extracts were reconstituted in DMSO to a final concentration of 5 mg/ml, followed by a two-fold serial dilution in a 96-well microtiter plates to achieve a series of concentrations. Dimethyl sulfoxide was used as negative control and isoniazid was a positive control. The plates containing M. smegmatis were incubated for 72 hours at 37˚C, thereafter 20 µL of 0.002% of resazurin was added and further incubated for 4 hours. Growth inhibition was indicated by a consistent blue resazurin colour, while presence of viable microorganisms was indicated by a pink colour (Farkas et al., 2018).

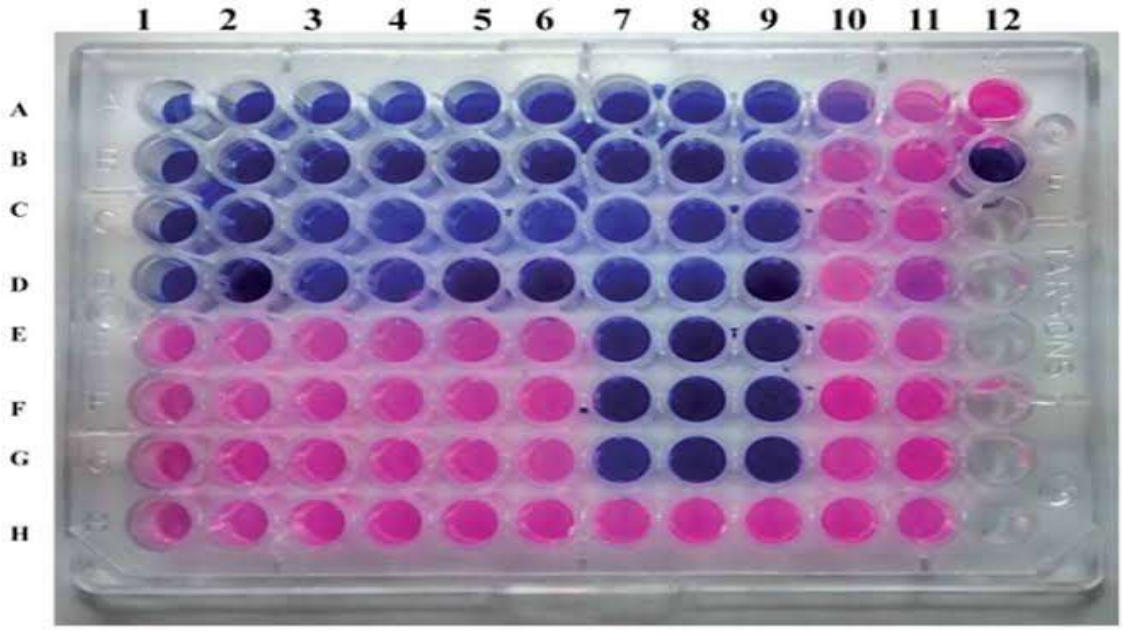


Figure 3.1: An illustration of the MIC microtiter plate after addition of resazurin. Blue indicating positive antimicrobial activity, and pink indicates potential resistance of the microorganisms. Adapted from https://www.researchgate.net/figure/Resazurin-Microtitre-Assay-REMA-for-determining-the-MIC-of-vasicine-against-one-of-the-fig2_282335785/amp on 17 November 22.

3.10 DATA ANALYSIS AND STATISTICAL ANALYSIS

Each experiment was at least performed in triplicate. All data shown in the tables and figures were quantified using Microsoft Excel, ImageJ, Origin 2023; and data were subjected to t-test, statistical significance was considered at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 EXTRACTION

The plant compounds were extracted using two solvents with different polarities, distilled water (polar/ more polar) and absolute methanol (non-polar). Figure 4.1 shows the yield of extraction, where methanol was most effective with the highest yield. Methanol has the capability of extracting most phytochemical compounds that are anti-inflammatory and antimicrobial (Nigussie et al., 2021; Truong et al., 2019). Some of the phytochemical constituents such as phenolic compounds (Tamilarasi & Meena, 2020) play a role in the formation nanoparticles by acting as reducing agents (Huong and Thang, 2020).

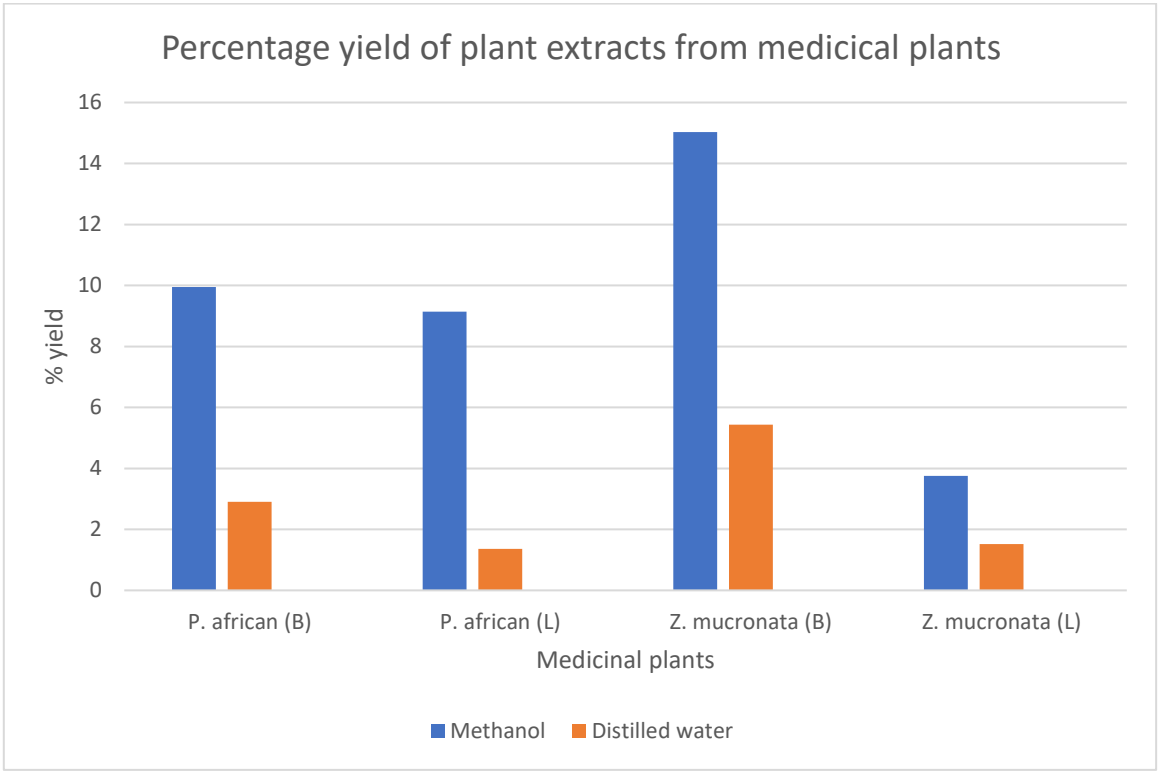
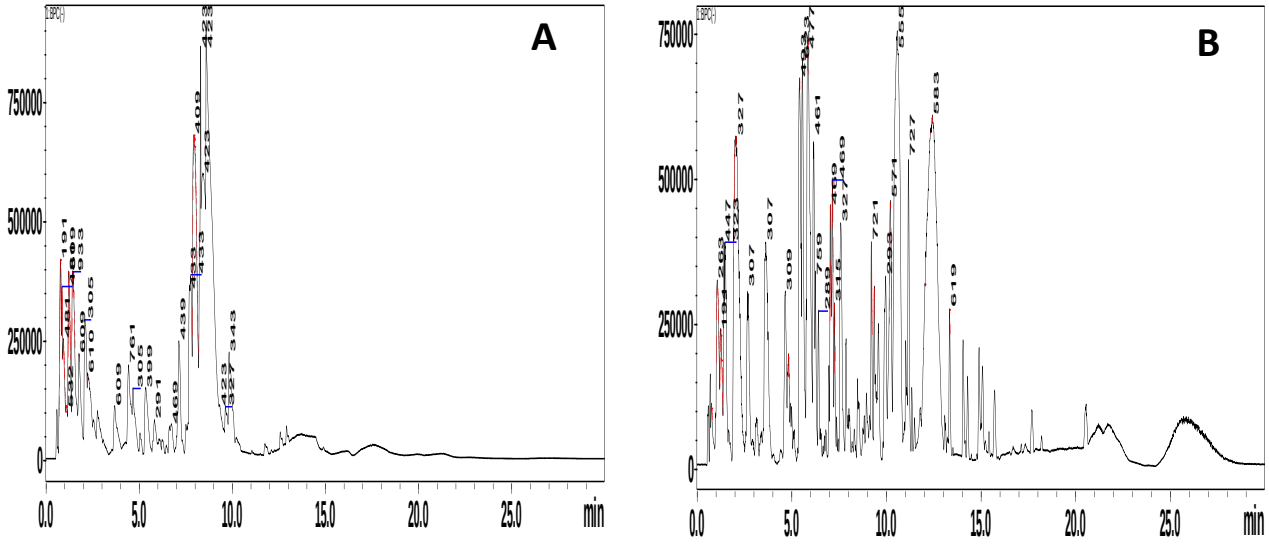


Figure 4.1: Percentage of the mass obtained after macerating powdered plants using absolute Methanol and distilled water for 78 hours.

4.2 LCMS ANALYSIS

The results obtained after analysis with the LC-MS are shown in Table 4.1 and Figure 4.2. The representation of base peak intensity chromatograms in Figure 4.2 indicate that these plants are phytochemically rich, containing a wide array of metabolites; as is shown by the separation of metabolites in methanol extracts of *P. africanum* and *Z. mucronata*, indicating differences in metabolite profiles of these species. It is also observed that the leaves (chromatogram B and D) have more compounds compared to the bark parts (chromatogram A and C). The tables below (Table 4.1 and Table 4.2) show that the leaves contain phytochemical compounds that have biological significance to human health, and the leaves are the only part reported to have anti-tubercular activity (Lawal et al., 2014).



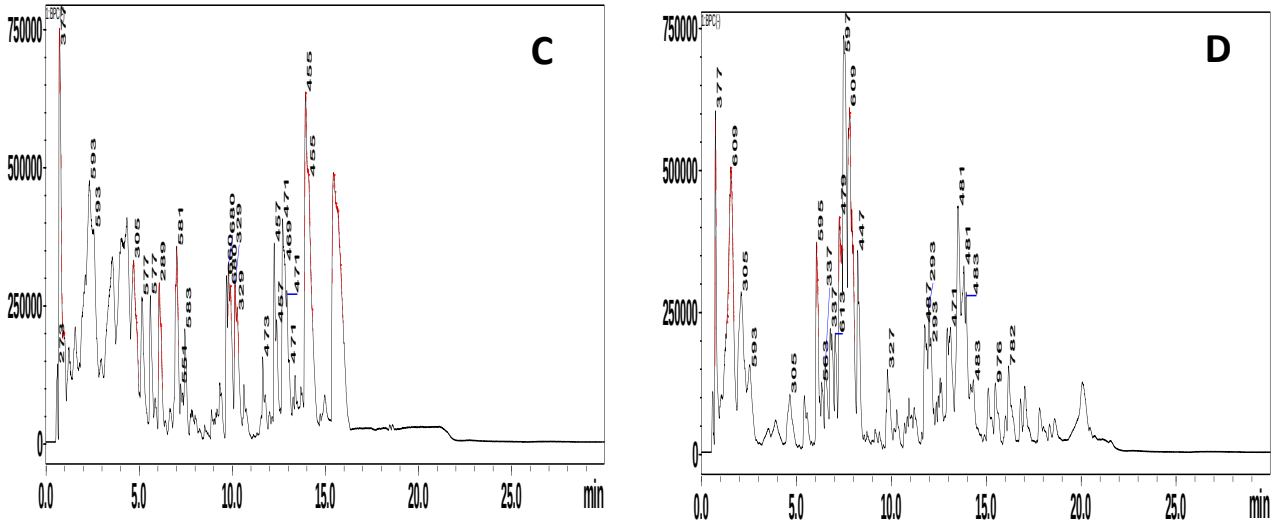


Figure 4.2: Chromatographs of compounds present in (A) *P. africanum* bark, (B) *P. africanum* leaves, (C) *Z. mucronata* bark, and (D) *Z. mucronata* leaves.

4.2.1 PELTOPHORUM AFRICANUM

Some studies have reported that *P. africanum* contains a variety of phytochemical compounds or secondary metabolites that are beneficial to human health. Mazimba (2014) showed that the plant has phytochemicals in all parts of the plants, e.g., catechin, epigallocatechin-3-O-gallate, and betulinic acid in leaves and flowers; bergenin, kaempferol, quercetin, myricetin and rutin in bark and flowers; and red colored gallotannin in roots and bark. In this study, the phytochemicals were profiled based on their biological activity and mostly their effects on symptoms of tuberculosis disease. Table 4.1 shows some of the phytochemical compounds after analyzing using liquid chromatography mass spectrometry.

Table 4.1: The compounds present in the *P. africanum* plant, determined by using global natural products social molecular networking (GNPS) software.

Compound name	Part	Phytochemical class	Biological activity	Reference
Epicatechin gallate	Bark	Flavonoids	Antioxidant Antineoplastic agent	Martin et al., 2021
Catergen	Bark	Flavonoids	Antioxidant	https://www.xmri.com/resource-

				center/dictionary.html?term=21422 25 October 2022
Bergenin	Bark Leaves	Polyphenols	Antarthritic Immunomodulatory Antidiabetic Osteogenic Wound-healing effects Anti-inflammatory Anti-HIV Anti-plasmodial Antioxidant	Hou et al., 2019 Kumar et al., 2019 Singh et al., 2017 Wang et al., 2017 Bajracharya, 2015 Jain et al., 2014 Mukherjee et al., 2013 Veerapur et al., 2012
Procyanidin B1	Bark	Polyphenol flavonoids	Inhibits hepatitis c virus RNA replication Exhibit anti-inflammatory effects	Li et al., 2010
Procyanidin C1	Bark	Tannins	Antioxidant Senotherapeutic activity	Xu et al., 2021
Cianidanol	Bark	Flavonoids	Antioxidant	Aronson 2016
Quercetin 3-O-glucuronide	Leaves	Flavonoids	Antioxidant Antidepressant	Guo et al., 2013
Rutin	Leaves	Flavonoids	Antioxidant Antibacterial Anti-inflammatory Antiprotozoal Antiplasmodic Antihypertensive Antiviral	Patel and Patel, 2019
Kaempferol	Leaves	Flavonoids	Anti-inflammatory Anti-cancer Antioxidant	Rho et al., 2011
Luteolin	Leaves	Flavonoids	Inhibits angiogenesis Induce apoptosis Reduce tumor growth in-vivo	Lopez-Lazaro 2009

			Chemotherapeutic Chemopreventive potential	

4.2.2 ZIZIPHUS MUCRONATA

Ziziphus mucronata is scientifically known to possess anti-inflammatory, antioxidant and antibacterial properties (Sameera and Mandakini, 2015; Najafi, 2013). It was reported that drinking a concoction of the whole plant boiled in water for three days, can treat bronchitis, fever, loss of appetite, anemia, weakness of the body, and liver problems (Mongalo et al., 2020; Koeven, 2001). Table 4.2 indicates the phytochemicals that may play a role in treatment of these diseases.

Table 4.2: The results obtained after analysis with the LC-MS and using GNPS to determine putative annotations. Compounds detected in *Z. mucronata* and their biological activities are shown below.

Compound name	Part	Phytochemical class	Biological activity	Reference
Catechin	Bark Leaves	Polyphenols	Antioxidant Antiviral Antimicrobial Anti-inflammatory Anti-allergenic Anti-cancer	Bae et al., 2020
Hydroquinidine	Bark	Alkaloids	Inhibits actions of parasympathetic nervous system Anti-arrhythmia agent Maintenance of sinus rhythm	Hermida et al., 2004
Canrenone	Bark Leaves	Steroids	Diuretic	https://pubchem.ncbi.nlm.nih.gov/compound/Canrenone#:~:text=Canrenone%20is%20an%20aldosterone%20antagonist,excretion%20a

				nd%20inhibiting%20potassium%20excretion 05 December 2022
Quercitrin	Leaves	Flavonoids	Antioxidant Protects neurovasculature structure in skin caused by oxidative stress Anti-inflammatory	Tang et al., (2019)
Eriodictyol-7-O-glucoside	Bark Leaves	Flavanones	Protects against cisplatin-induced toxicity Antioxidant Anti-inflammatory	Tomaino et al., 2010
Hyperoside	Bark Leaves	Flavanol glycosides	Anti-inflammatory Antioxidant Anti-diabetic Anti-thrombotic	Shukla et al., 2019
Isorhamnetin 3-galactoside	Leaves	Flavonoids	Antioxidant Anti-inflammatory Neuroprotective	Kim et al., 2013
Theaflavin	Bark	Polyphenols	Antioxidant Anti-inflammatory Anti-bacterial Anti-cancer Nephroprotective	Rohini et al., 2018
Myricetin 3-O-rutinoside	Bark Leaves	Flavonoids	Antibacterial Antioxidant Anticarcinogenic	https://pubmed.ncbi.nlm.nih.gov/compound/44259428 05 December 2022

Rutin	Leaves	Flavonoids	Antioxidant Antibacterial Anti-inflammatory Antiprotozoal Antiplasmodic Antihypertensive Antiviral	Patel and Patel, 2019
Procyanidin B1	Bark and leaves	Polyphenol flavonoids	Inhibits hepatitis C virus RNA replication Exhibit anti-inflammatory effects	Li et al., 2010
Cianidanol	Bark	Flavonoids	Antioxidant	Aronson 2016
Procyanidin C1	Bark Leaves	Tannins	Antioxidant Senotherapeutic activity	Xu et al., 2021

4.3 SYNTHESIS AND CHARACTERIZATION OF NANOPARTICLES

4.3.1 SYNTHESIS OF NANOPARTICLES

The initial confirmation of formation of the gold and silver nanoparticles is by visually observing the changes in color of the solution. Plants have enzymes, proteins or phytochemicals that aid in the reduction of metal ions that result in formation of metal nanoparticles (El-Borady et al., 2020; Priya and Priya, 2020; Islam et al., 2019; Vijaya et al., 2019; Rajan et al., 2015). Figure 4.3 shows the color change after 24-hour incubation. The Eppendorf tubes have different shades of brown (A) and purple (B), which indicates that the darker the solution, the more nanoparticles formed (Tamilarasi and Meena, 2020).

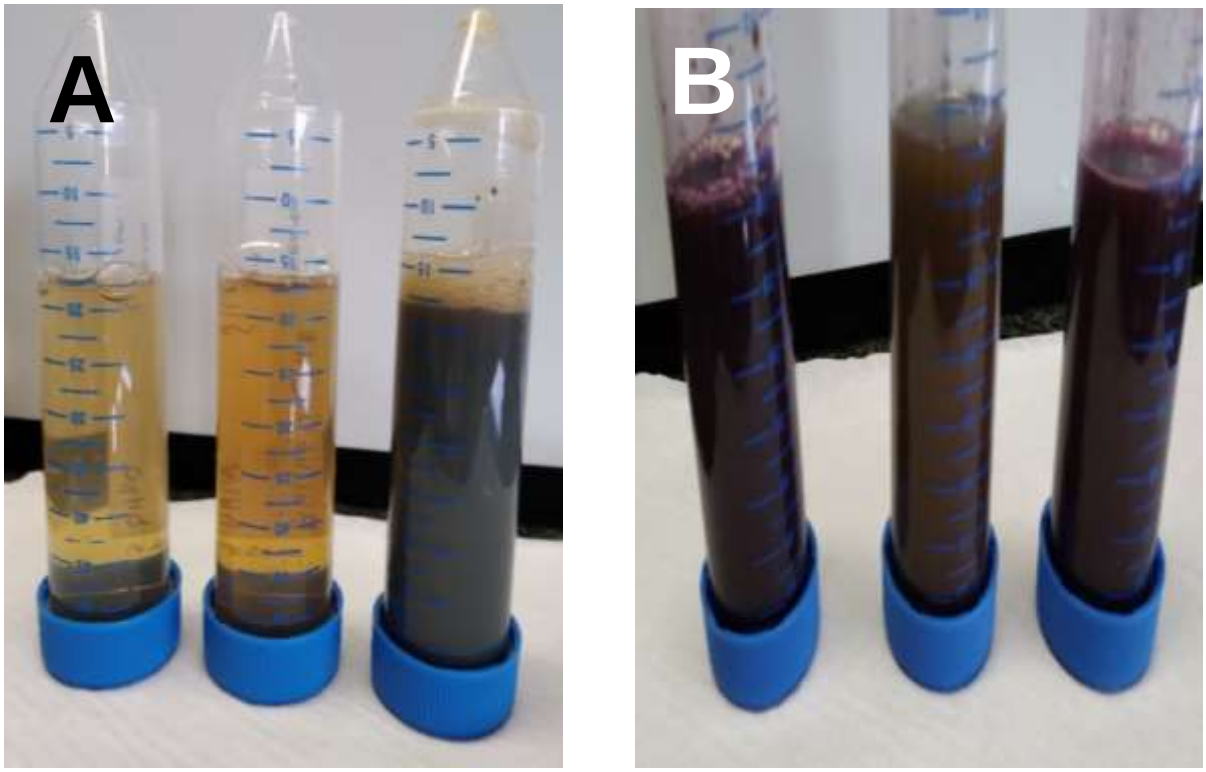


Figure 4.3: Solutions of (A) silver and (B) gold nanoparticle synthesized using different plants of *P. africanum* and *Z. mucronata* after 24 hours.

4.3.2 TRANSMISSION ELECTRON MICROSCOPY

The nanoparticle sizes and distribution were determined by using transmission electron microscope. Transmission electron microscopes operate on the principle of imaging structural features and crystalline structures, by shining a high energy beam of electrons through thin sample. Collectively, the nanoparticles synthesized in this study were clustered, and few were scattered (Figure 4.4). As shown in the histogram figures, majority of the nanoparticles, both gold and silver nanoparticles, were generally small, with sizes ranging from 5 nm up to 30 nm. Large nanoparticles were limited, ranging up to 180 nm in sizes. The gold nanoparticles have inconsistent shapes, PMB(g) formed oval, hexagonal and triangular shaped nanoparticles, whereas ZML(g) and ZMB(g) were oval. Silver nanoparticles were unusual shapes for both ZMB(s) and PHB(s) nanoparticles.

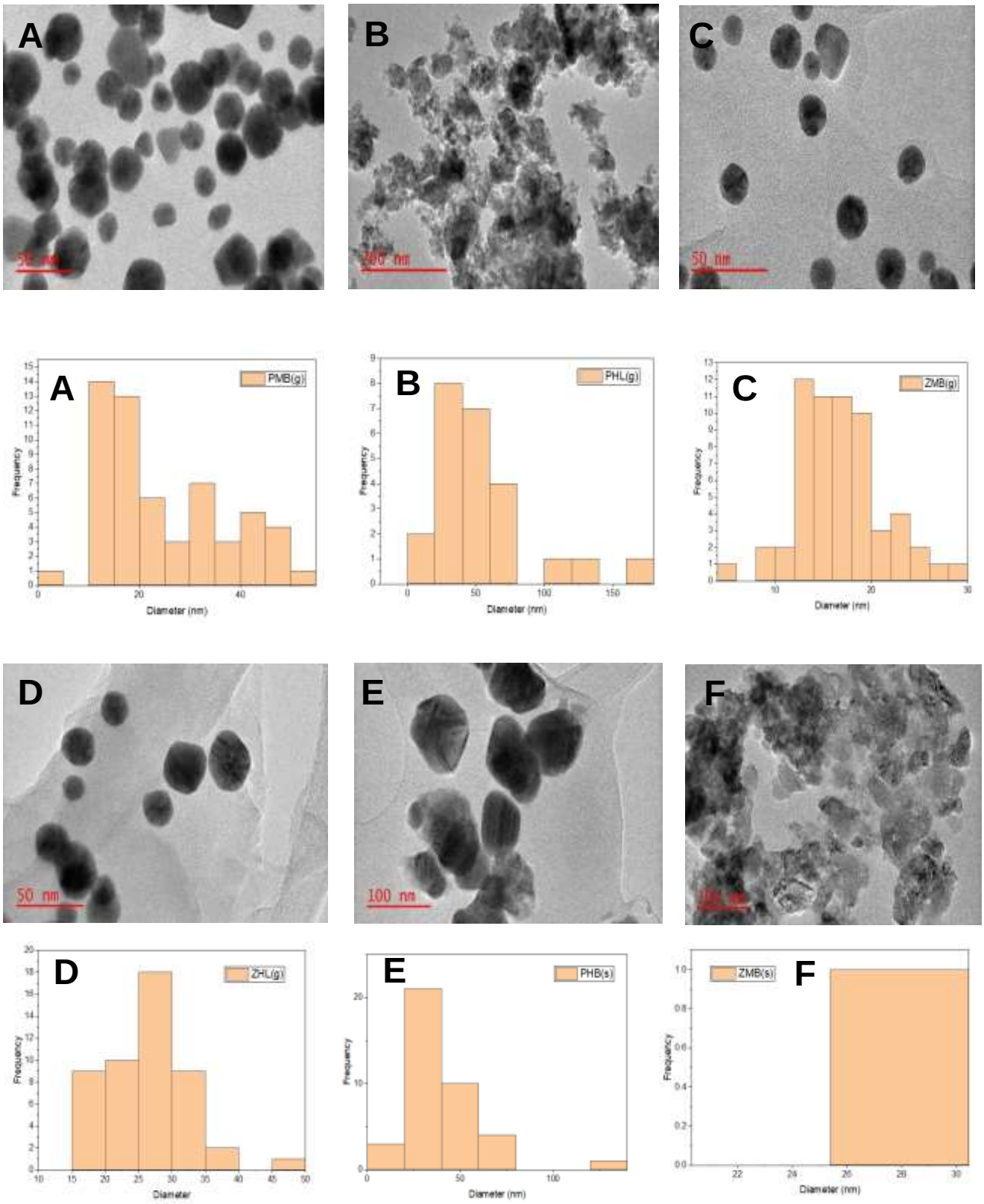
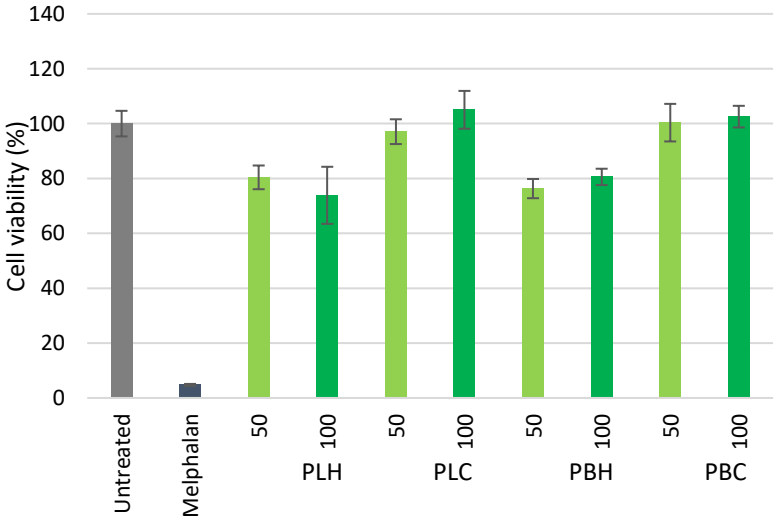


Figure 4.4: Images of the silver and gold nanoparticles viewed under a transmission electron microscopy; and histogram plots illustrating sizes of gold nanoparticles (a. PMB, b. PHL, c. ZMB, d. ZHL) and silver nanoparticles (e. PHB, f. ZMB) viewed under transmission electron microscope.

Aspects such as size, shape, and distribution, are dependent on the reducing agents or phytochemical compounds from the medicinal plants used in the synthesis (Marslin et al., 2018; Baharara et al., 2014). Nanoparticle sizes, distribution and shapes affect the biological activities of the nanoparticles. The sizes and surface chemistry properties of nanoparticles influence the entry and penetration of nanoparticles into pathogenic microorganisms or diseased cells (El-Rafie et al., 2013). After entry into the cells, the nanoparticles bind with merpapto groups of bacterial proteins, causing inhibition of DNA replication and lead to cell death (Li et al., 2018).

4.4 CYTOTOXICITY

The Vero cell line was used to carry out the in vitro cytotoxicity assay. The results obtained show the cell viability after treatment with plant extracts, gold, and silver nanoparticles (Figures 4.5 - 4.6). Collectively, the plant extracts and gold nanoparticles exhibited relatively low cytotoxic activity on the Vero cells compared to the silver nanoparticles at both 50 µg/mL and 100 µg/mL concentrations.



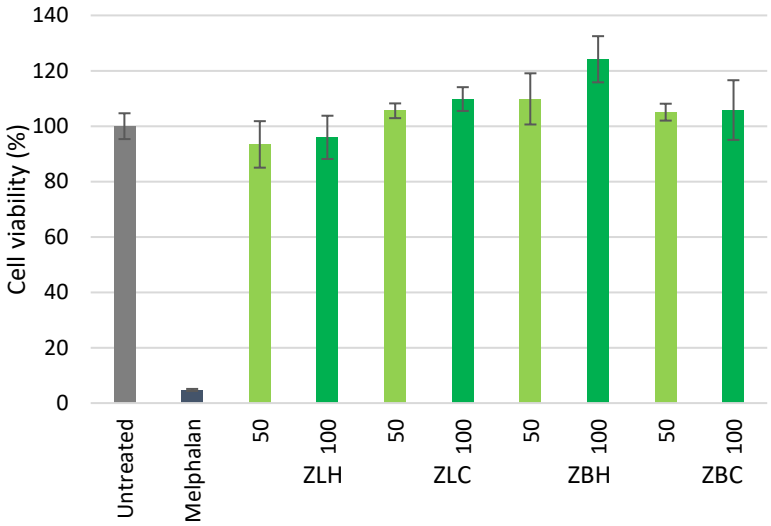
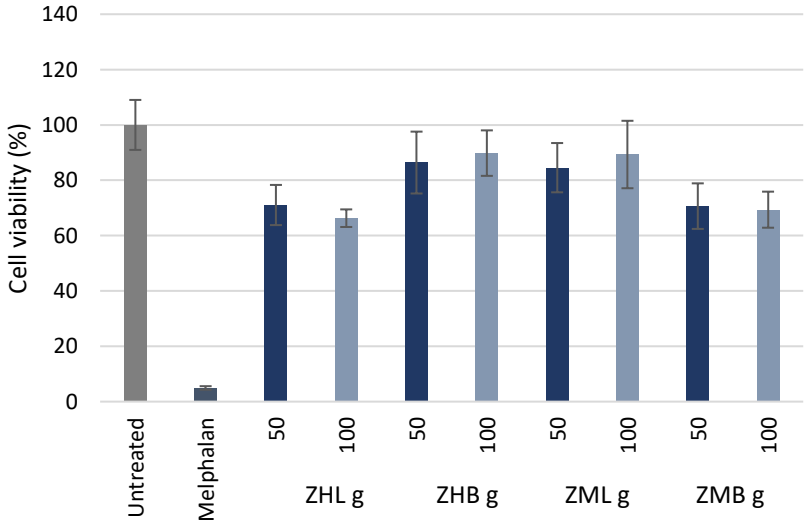


Figure 4.5: Cytotoxicity of 8 plant extract samples in Vero cells after 48 hours of treatment. Melphalan (30 µM) was used as positive control. Error bars indicate standard deviation of quadruplicate values obtained from a single experiment.

The silver nanoparticles of PHL, PHB, ZML and ZMB exhibited significant cytotoxicity at both treatment concentrations [p-values were (0.0035; 0.0011), (0.00006; 0.0004), (0.000004; 0.000002), (0.00002;0.00002) respectively), while ZHL showed toxicity at concentration of 100 µg/mL. A study done by Speshock et al (2010), reported that silver nanoparticles significantly reduced the viability on Vero cells at 75 to 100 µg/mL after 48 hours of incubation. Another study performed by Hussein et al (2020), showed that 100 µg/mL concentrations of silver nanoparticles exhibited significant cytotoxic activity on Vero. It can be concluded that silver nanoparticles can be harmful to human normal cells at concentrations ≥50 µg/mL.



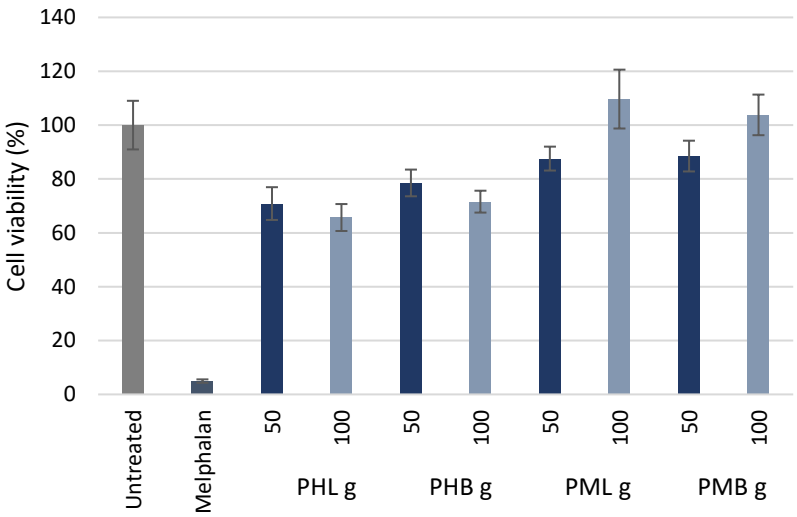
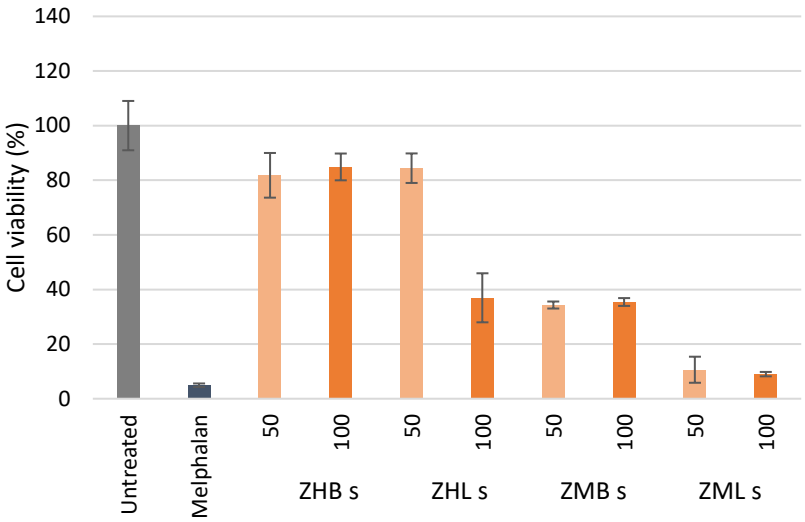


Figure 4.6: Cytotoxicity of 8 Gold (g) nanoparticle samples in Vero cells after 48 hours of treatment. Melphalan (30 µM) was used as a positive control. Error bars indicate standard deviation of quadruplicate values obtained from a single experiment.



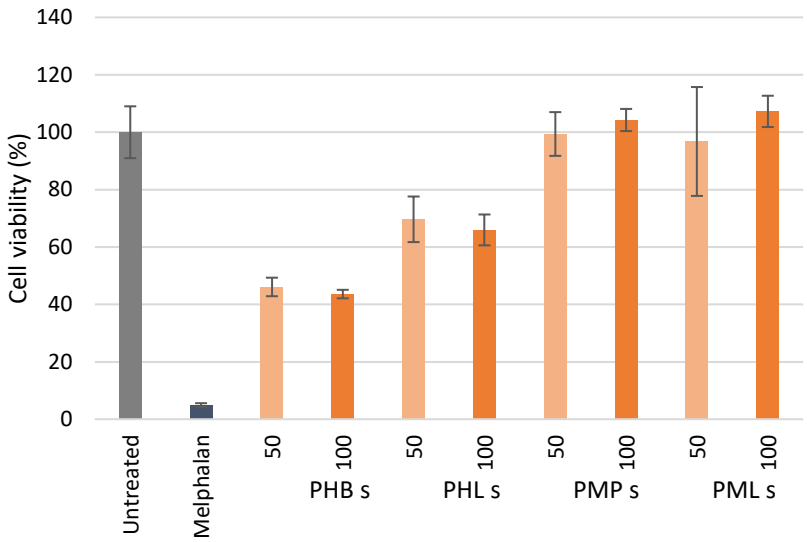


Figure 4.7: Cytotoxicity of 8 Silver (s) nanoparticle samples in Vero cells after 48 hours of treatment. Melphalan (30 µM) was used as a positive control. Error bars indicate standard deviation of quadruplicate values obtained from a single experiment.

4.5 ANTI-INFLAMMATORY ACTIVITY

4.5.1 ANTI-INFLAMMATORY SCREENING

The anti-inflammatory activity of plant extracts, gold and silver nanoparticles were evaluated using the Griess assay and RAW 264.7 macrophages. The cytotoxic effect of sample treatment on the RAW cells was determined to accurately establish potential anti-inflammatory effect. A decrease in nitrite concentration in response to LPS activation of RAW 264.7 macrophages without effecting cell viability, as seen with cells treated with aminoguanidine (AG), indicates anti-inflammatory activity. In this study, the anti-inflammatory activity of Z. *mucronata* methanol extracts were not considered due to the cytotoxicity at both 50 and 100 µg/mL concentrations.

Silver nanoparticles of ZML ($p \leq 0.0000015$) and ZHL ($p \leq 0.00033$), and gold nanoparticles and plant extracts of PML ($p \leq 0.0000011$ and $p \leq 0.000016$ respectively) exhibited anti-inflammatory activity at 100 µg/mL, whereas PML was able to decrease nitrite concentration at both concentrations. The *P. africanum* plants contains numerous flavonoids that have been reported to have anti-inflammatory activities as reported in Table 1.

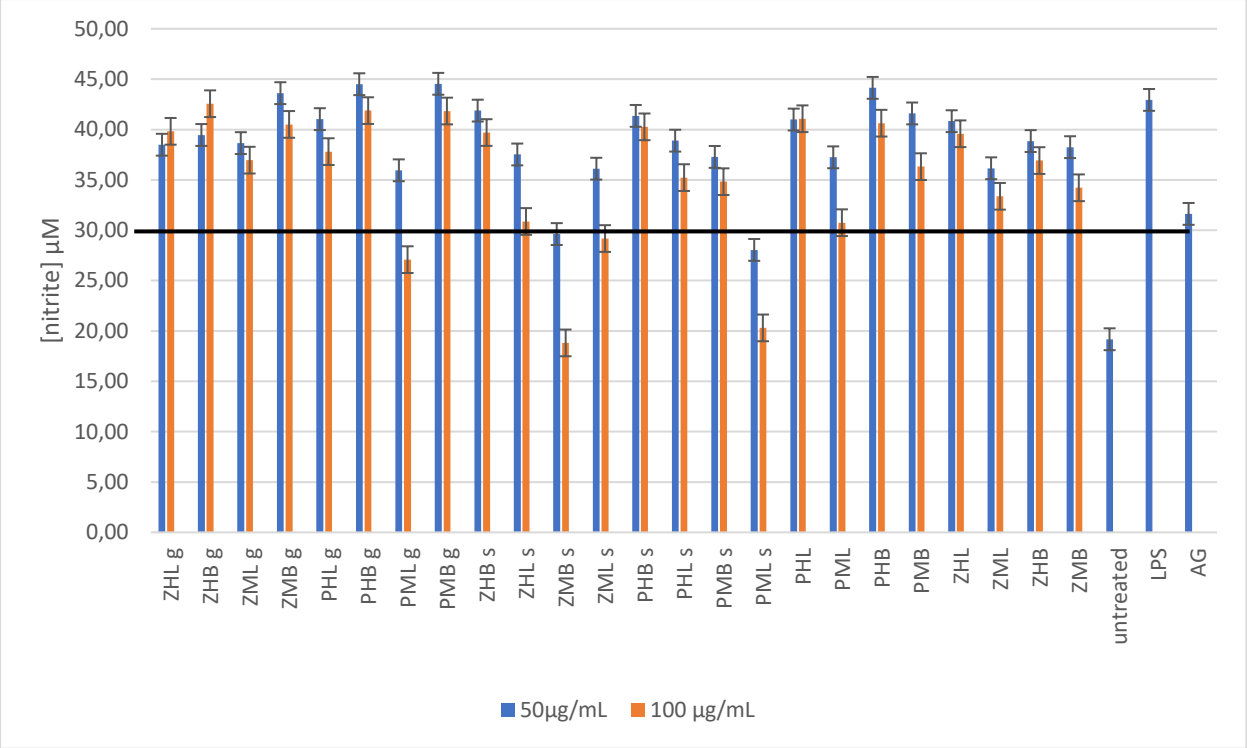


Figure 4.8: NO production in LPS activated macrophages treated with different concentrations of samples as indicated in the Figure. “g” indicates gold nanoparticles and “s” indicates silver nanoparticles. Bar graph represents quadruplicate values of one experiment. Error bars represent the standard deviation of the mean. (Z: *Ziziphus mucronata*; P: *Peltophorum africanum*; M: Methanol extract; H: Distilled water extract; L: Leaves; B: Bark).

4.5.2 VIABILITY SCREENING

To evaluate the viability of cells following exposure of the RAW 264.7 cells to the plant extracts, gold and silver nanoparticles, the cells were exposed to 50 µg/mL and 100 µg/mL of compound samples. The viability of the cells was based on mitochondrial dehydrogenase’s formation of formazan (MTS assay) (Alsaleh et al., 2019). The data (Figure 4.9) obtained after exposure shows that compound samples are not associated with reduction of cell viability at both concentrations, except silver nanoparticles of ZMB that majorly reduced the cell viability. Thus, the silver nanoparticles of ZMB were disregarded for anti-inflammatory analysis due to their toxicity to the cells.

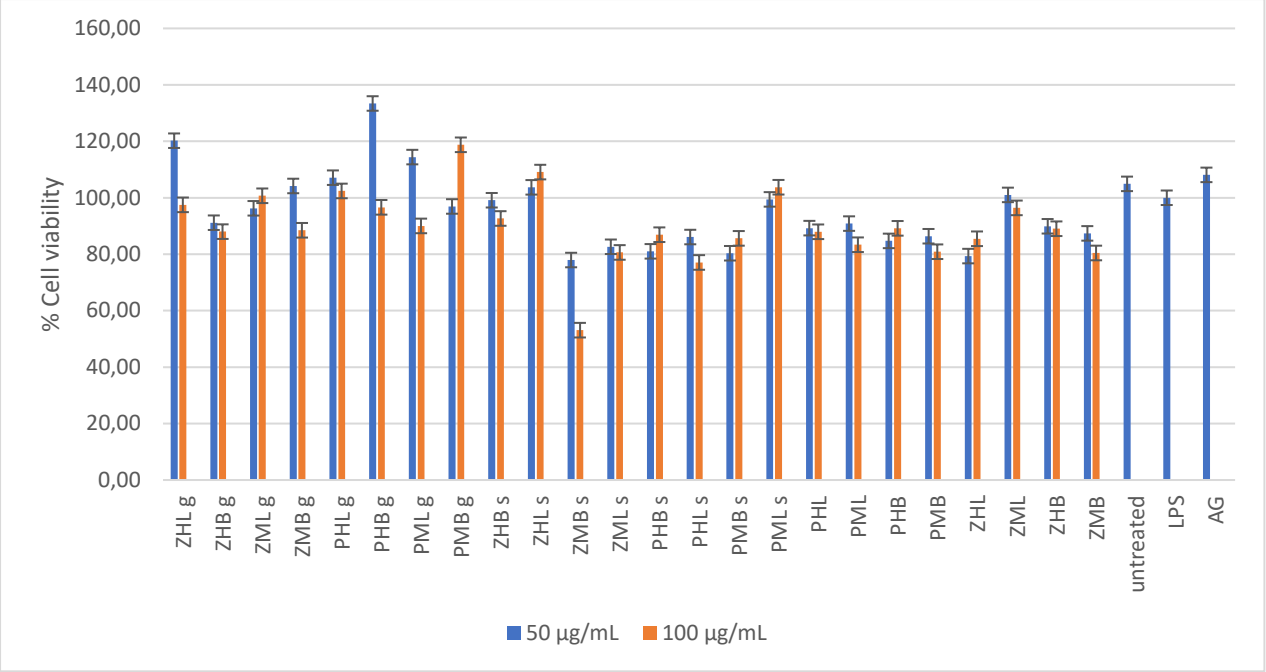


Figure 4.9: Cell viability (%) of LPS activated macrophages after 24 hours pf exposure to treatments. “g” indicates gold nanoparticles and “s” indicates silver nanoparticles. Bar graph represents quadruplicate values of one experiment. Error bars represent the standard deviation of the mean. (Z: *Ziziphus mucronata*; P: *Peltophorum africanum*; M: Methanol extract; H: Distilled water extract; L: Leaves; B: Bark).

4.6 ANTI-MYCOBACTERIAL ACTIVITY

The minimum inhibitory concentration assay was used to determine the lowest concentration of sample compounds active against M. smegmatis. Table 4.3 shows the sample compounds that exhibit antimycobacterial activity. Plant extracts (PHB, PMB, ZMB and ZHL) and silver nanoparticles (ZML, ZHB, ZML and ZMB) showed activity; gold nanoparticles had little to no activity against M. smegmatis. *P. africanum* was reported to possess phytochemicals such as flavanols, benzenoids, condensed flavonoids in the bark part (Mazimba, 2014) that have antioxidant and antibacterial activity (Ramabulana et al., 2021). As shown in Table 4.1, *P. africanum* and *Z. mucronata* contains significant number of flavonoids that can act against the *M. smegmatis*.

Table 4.3: Antimycobacterial activity screening of plant extracts, silver, and gold nanoparticles at 5 mg/ml concentration.

Samples (mg/ml/)	Active	Inactive
PHL		X
PHB	X	
PML		X
PMB	X	
ZHL	X	
ZHB		X
ZML		X
ZMB	X	
PHL(g)		X
PHB(g)		X
PML(g)		X
PMB(g)		X
ZHL(g)		X
ZHB(g)		X
ZML(g)		X
ZMB(g)		X
PHL(s)		X
PHB(s)		X
PML(s)		X
PMB(s)		X
ZHL(s)	X	
ZHB(s)	X	
ZML(s)	X	
ZMB(s)	X	

Silver nanoparticles have been reported to have the ability to attach and enter cells due to their small sizes (Koduru et al., 2018). In this study, the silver nanoparticles ranged up to 30 nm. In a study by Praba et al. (2013), they synthesized silver nanoparticles chemically and tested against *M. tuberculosis* H37Rv (ATCC 27294) and *M. smegmatis* mc2155 strains. It was found that the silver nanoparticles had no effect on the strains, however, they inhibited growth of the organism after treatment with chloroform. Since this current study synthesized nanoparticles using medicinal plants, it is proof that plants enhance biological activity of nanoparticles.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study was aimed to evaluate the efficacy of silver and gold nanoparticles capped with selected medicinal plants against tuberculosis. The secondary objectives were derived to achieve the primary objective.

The first secondary objective was to profile phytochemical compounds from the medicinal plants using liquid chromatography mass spectroscopy (LC-MS). The plants had abundant antioxidant agents that played a major role in the synthesis and biological activity of the silver and gold nanoparticles.

Secondly, the synthesis of silver and gold nanoparticles using green technology was done. The nanoparticles were synthesized successfully. A color change from clear to brown for silver nanoparticles, and grape-purple for gold nanoparticles, was observed after 24 hours; indicating positive nanoparticle formation.

Thirdly, the nanoparticles were characterized using transmission electron microscopy (TEM). The shapes for gold nanoparticles were oval, hexagonal, and triangular; whilst silver nanoparticles had irregular shapes. The nanoparticles were analyzed using software to determine the sizes.

The fourth objective was to determine the toxicity of the compound samples using MTT assay on Vero cells. Gold nanoparticles and plant extracts were not toxic to the cells; silver exhibited toxic activity of the cells at 50 and 100 µg/mL.

The fifth objective was to determine anti-inflammatory activity on RAW 264.7 cells. Silver nanoparticles of ZML and ZHL, and gold nanoparticles and plant extracts of PML exhibited anti-inflammatory activity at 100 µg/mL, whereas PML was able to decrease nitrite concentration at both concentrations.

Finally, the sixth objective was to evaluate antimycobacterial activity on *M. smegmatis*. Plant extracts (PHB, PMB, ZMB and ZHL) and silver nanoparticles (ZML, ZHB, ZML and ZMB) showed activity; gold nanoparticles had little to no activity against *M. smegmatis*.

This study showed that the selected plants were a good source for nanoparticle synthesis. The transmission electron microscope (TEM) images were useful in proving the formation, size, shape, and distribution of the nanoparticles. The efficacy of some silver nanoparticles on M. smegmatis showed that they can render antimycobacterial efficacy and strengthen medicinal value of plants. The MTT assay showed that gold nanoparticles safer compared to silver nanoparticles, which exhibited toxic activity on the cells. Yet the silver nanoparticles were able to decrease nitrite production. Although the silver nanoparticles had good anti-inflammatory and antimycobacterial, they are unsafe for human consumption.

5.2 LIMITATIONS

Due to unforeseen circumstances (availability of equipment, loadshedding), the nanoparticles were not analyzed using FTIR and X-ray to determine the chemical compositions.

5.3 RECOMMENDATION

Most of the nanoparticles did not have activity on *M. smegmatis*. Testing the silver and gold nanoparticles on clinical strains of *M. tuberculosis* may be essential to observe the antimycobacterial effects.

REFERENCES

Alexander, K.A., Laver, P.N. and Michel, A.L., (2010). Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. *Emergence of Infectious Diseases,* 16(8): 1296-1299

Akpalo, A.E., Saloufou, I.K., Eloh, K. and Kpegba, K., (2020). Wound healing biomolecules present in four proposed soft aqueous extraction of *Ageratum conyzoides* Linn. *International Journal of Biological and Chemical Sciences*,14(2): 938-651

Alsaleh, N.B., Minarchick, V.C, Mendoza, R.P., Sharma, B., Podila, R. and Brown, J.M., (2019). Silver nanoparticle immunomodulatory potential in absence of direct cytotoxicity in RAW 264.7 macrophages and MPRO 2.1 neutrophils. *Journal of Immunotoxicology*, 15(1): 63-73

Anisimova, Y.V., Gelperina, S.I., Peloquin, C.A. and Heifets, L.B., (2000). Nanoparticles as antituberculosis drugs carriers: effects on activity against *Mycobacterium tuberculosis* in human monbocyte-derived macrophages. *Journal of Nanoparticle Research*, 2: 165-171

Anupam, R., Onur, B., Sudip, S., Mandal, A.K. and Yilmaz, M.D., (2019). Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RCS Advances,* 2019(5)

Aronson, J.K., (2016). The history of Meyley's side effects of drugs and the side effects of drugs. *Meyley's side effects of drugs*, 16th edition

Bae, J., Kim, N., Shin, Y, Kim, S. and Kim, Y., (2020). Activity of catechins and their applications. *Biomedical Dermatology*, 4(8)

Baharara, J., Namvar, F., Ramezani, T., Hosseini, N. and Mohamad, R., (2014). Green synthesis of silver nanoparticles using *Achillea biebersteinii* flower extract and its anti-angiogenic properties in the rat aortic ring model, *Molecules,* 19 (2014) 4624–4634

Bajracharya, G.A., (2015). Diversity, pharmacology and synthesis of bergenin and its derivatives: Potential materials for therapeutic usages. *Fitoterapia*, 101: 133-152

Caminero, J.A.L., Mendoza, G.P. and Castro, F.R., (2021). Multi-drug resistant tuberculosis, ten years later. *Medicina Clinica*, 156(2021): 393-401

Cheng, H.Y., (2006). Ent-Epiafzelechin-(4 ->8)-epiafzelechin extracted from *Cassia javanica* inhibits herpes simplex virus type 2 replication. *Journal of Medical Microbiology*, 55: 201-206

Chinsembu, K.C., (2016). Ethnobotanical study of medicinal flora utilised by traditional healers in the management of sexually transmitted infections in Sesheke District, Western Province, Zambia. *Revista Brasileira de Farmacognosia*, 26: 268-274

Chinsembu,K.C., Syakalima, M. and Semenya, S.S., (2019). Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Lusaka, Zambia. *South African Journal of Botany*, 122(2019): 380-384

Das, K., Tiwari, R.K.S. and Shrivastava, D.K., (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research,* 4(2):104-111

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N., (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(4): 654-660

Forrellad, M.A., Klepp, L.I. and Gioffré, A., (2013). Virulence factors of the *Mycobacterium tuberculosis* complex. *Virulence,* 4(1):3-66

Echeverria-Valencia, G., Flores-Villalva, S. and Espitia C.I., (2018). Virulence factors and pathogenicity of *Mycobacterium*. In: Ribón W editor. *Mycobacterium - Research and Development*. *In Tech*, 231-255

Eddouks, M., Lemhadri, A., Hebi, M., Hidani, A.E.L., Zeggwagh, N.A., Bouhali, B.E.L., Hajji, L. and Burcelin, E., (2012). *Capparis spinosa L.* aqueous extract evokes antidiabetic effect in streptozotocin-induced diabetic mice. *Journal of Phytomedicine*, 7(2): 191-198

El-Borady, O.M., Ayat, M.S., Shabrawy, M.A. and Millet, P., (2020). Green synthesis of gold nanoparticles using parsley leaves extract and their applications as an alternative catalytic, antioxidant, anticancer, and antibacterial agents. *Advanced Powder Technology*, 31: 4390-4400

El-Rafie, H., El-Rafie, M. and Zahran, M., (2013). Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. *Carbohydrate Polymer,* 96: 403–10

Gerszten, P.C., Gerszten, E. and Allison, M.J., (2001). Diseases of the spine in South American mummies. *Neurosurgery,* 48(1): 208-213

Govind, D., (2011). Neutral to target emotion conversion using source and suprasegmental information. Interspeech, 20: 2969-2972

Guo, X.D., Zhang, D.Y., Gao, X.J., Parry, J., Liu, K., Liu, B.L. and Wang, M., (2013). Quercetin and quercetin-3-O-glucuronide are equally effective in ameliorating endothelial insulin resistance through inhibition of reactive oxygen species-associated inflammation. *Molecular Nutrition & Food Research*, 57(6): 1037-1045

Halliwell, B., Aeschbach, R., Loliger, J. and Aruoma, I.O., (1981). The characterization of antioxidants. *Pergamon*, 33(7): 601-317

Hermida, J.S., Denoy, I., Clerc, J., Extramiana, F., Jarry, G., Miiliez, P., Guicheney, P., Fusco, S.D., Rey, J.L., Cauchemez, B. and Leenhardt, A., (2004). Hydroquinidine therapy in Brugada syndrome. *Journal of the American College of Cardiology*, 43(10): 1853-1860

Hou, W., Ye, C., Chen, M., Li, W., Gao, X., He, R., Zheng, Q. and Zhang, W., (2019). Bergenin activates SIRT1 as a novel therapeutic agent for osteogenesis of bone mesenchymal stem cells. *Frontiers in Pharmacology*, 10: 618

Huong, V.T.L. and Thang, (2020). Green synthesis, characterization and antibacterial activity of silver nanoparticles using *Sapindus mukorossi* fruit pericarp extract. *Materials Today: Proceedings*

Islam N.U., Jalil, K., Shahid, M., Rauf, A., Muhammad, N., Khan, A., Shah, M.R. and Khan, M.A., (2019). Green synthesis and biological activities of gold nanoparticles functionalized with *Salix alba*. *Arabic Journal of Chemistry*, 12: 2914-2925

Jain, S. K., Singh, S., Khajuria, A., Guru, S. K., Joshi, P., Meena, S., Nadkarni, J.R., Singh, A., Bhushan, S., Bharate, S.B. and Vishwakarma, R.A., (2014). Pyrano-isochromanones as IL-6 inhibitors: synthesis, in vitro and in vivo antiarthritic activity. *Journal of Medical Chemistry*, 57(16): 7085-7097

Jibrin, Y.B., Ali, A.B., Saad, S.T. and Kolo, P.M., (2012). Prevalence of treatment failure among pulmonary tuberculosis patients in federal medical center, Gombe, Northeastern Nigeria. *Hindawi,* 2013: 1-5

Kaforou, M., Broderick, C., Vito, O., Levin, M., Scriba, T.J. and Seddon, J.A., (2022). Transcriptomics for child and adolescent tuberculosis. *Immunological Reviews*, 309(1): 97-122

Kalimuthu, K., Babu, R.S., Venkataraman, D., Bilal, M. and Gurunathan, S., (2008). *Colloids Surf, B.,* 65: 150-153

Kesharwani, P. (ed.), (2020). Nanotechnology based approaches for tuberculosis treatment. *Academic Press*, London, UK

Kiers A, Klarenbeek A, Mendelts B, Van Soolingen D, Koëter G., (2008). Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals. *International Journal of Tuberculosis Lung Diseases off Journal International Union of Tuberculosis Lung Disease*, 12(12): 1469-1473

Kim, T.H., Ku, S.K. and Bae, J.S., (2013). Anti-inflammatory activities of isorhamnetin-3-O-galactoside against HMGB1-indused inflammatory responses in both HUVECs and CLP-induced septic mine. *Journal of Cellular Biochemistry*, 114(2): 336-345

Koeven, E., (2001). Medicinal, poisonous and edible plants in Namibia. Windhoek (Namibia), *Klause Hess Publishers*, Namibia

Koduru, J., Kailasa, S., Bhamore, J., Kim, K., Dutta, T. and Vellingiri, K., (2018). Phytochemical-assisted synthetic approaches for silver nanoparticles antimicrobial applications: A review. *Advances in Colloid and Interface Science*, 256: 326-339

Kumar, S., Sharma, C., Kaushik, S. R., Kulshreshtha, A., Chaturvedi, S., Nanda, R. K., Bhaskar, A., Chattopadhyay, D., Das, G. and Dwivedi, V.P., (2019). The phytochemical bergenin as an adjunct immunotherapy for tuberculosis in mice. *Journal of Biological Chemistry*, 294: 8555-8563

Lall, N. and Meyer, J.J.M., (2001). Inhibition of drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. *Journal of Ethnopharmacol*ogy, 78: 213-216

Lawal, I.O., Grierson, D.S., and Afolayan, A.J., (2014). Phytotherapeutic information on plants used for the treatment of tuberculosis in Eastern Cape Province, South Africa. *Evidence Base Complimentary Alternative Medicine*, 11(2014).

Leonov, G., Kuznetsov, N. and Mokaev, T., (2015). Homoclinic orbits, and self-excited and hidden attractors in a Lorenz-like system describing convective fluid motion. *The European Physical Journal Special Topics*, 224(8): 1421-1458

Li., S., Kodama, E.N. and Inoue, Y., (2010). Procyanidin B1 purified from Cinnamomi cortex suppresses hepatitis C virus replication. *Antiviral Chemistry and Chemotherapy*, 20(6): 239-248

Li, W.R., Xie, X.B., Shi, Q.S., Zeng, H.Y., You-Sheng, O.Y., Chen, Y.B., (2018) Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Applied Microbiology Biotechnology*, 85:1115-1122.

Lopez-Lazaro, M., (2009). Distribution and biological activities of the flavonoid luteolin. *Mini-Reviews in Medicinal Chemistry*, 9(1): 31-59

Louw, J.H. and Scholes, M., (2002). Forest site classification and evaluation: A South African perspective. *Forest Ecology and Management.,*171: 153-168

Madisha, J.K., Aiyegoro, A.O. and Ashafa, A.O.T., (2016). Antimicrobial activities of selected plants used in the management of tuberculosis in Limpopo Province, South Africa. *South African Journal of Botany*, 103: 323

Maier, W., Khoza, T., Harmse, N., Wingfield, B.D. and Wingfield, M.J., (2006). A disease epidemic on *Zizyphus mucronata* in the Kruger National Park caused by *Coniodictyum chevalieri*. *Studies in Mycology*, 55: 279-288

Mann, A., Yahaya, Y., Banso, A. and John, F., (2008). Phytochemical and antimicrobial activity of *Terminalia avicennioides* extracts against some bacteria pathogens associated with patients suffering from complicated respiratory tract diseases. *Journal of Medicinal Plants Research*, 2(5): 94-97

Martin, C.R., Preedy, V.R., and Rajendram, R., (2021). Diagnosis, management and modeling of neurodevelopmental disorders. The neuroscience of development. *Elsevier*, 589-599

Mazid, M., Khan, T.A. and Mohammad, F., (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, 3(2): 232-249

McGaw, L. and Eloff, J.N., (2008). Ethnoveterinary use of southern Africa plants and scientific evaluation of their medicinal properties. *Journal of ethnopharmacology* 119 (3): 550 – 574

Mikhailova, E.O., (2021). Gold nanoparticles: biosynthesis and potential of biomedical application. *Journal of Functional Biomater,* 12(4): 70

Mohanty, S., Jena., P and Mehta, R., (2013). Cationic antimicrobial peptides and biogenic silver nanoparticles kill mycobacteria without eliciting DNA damage and cytotoxicity in mouse macrophages. *Anti-microbiological Agents Chemotherapy,* 57(8): 3688-3698

Mongalo, N.I., Mashele,S.S. and Makhafola, T.J., (2020). *Ziziphus mucronata* Willd. (*Rhamnaceae*): it's botany, toxicity , phytochemistry and pharmacological activities. *Heliyon*, 6(2020)

Montelongo-Peralta, L.Z., León-Buitimea, A., Palma-Nicolás, J.P., Gonzalez-Christen, J. and Morones-Ramírez, J.R., (2019). Antibacterial activity of combinatorial treatments composed of transition-metal/antibiotics against *Mycobacterium tuberculosis*. *Scientific Report,* 9(1):1-6

Mukherjee, H., Ojha, D., Bharitkar, Y. P., Ghosh, S., Mondal, S., Kaity, S., Dutta, S., Samanta, A., Chatterjee, T.K., Chakrabarti, S., Mondal, N.B. and Chattopadhyay, D., (2013). Evaluation of the wound healing activity of *Shorea robusta*, an Indian ethnomedicine, and its isolated constituent(s) in topical formulation. *Journal of Ethnopharmacology*, 149(1): 335-343

Najafi, S., (2013). Phytochemical screening and bacterial activity of leaf extract of *Ziziphus mauritiana* Lam*. International Research Journal of Applied Basic Sciences*, 4(2013): 3274-3276

Nardell, E. and Dharmadhikari. A., (2010). Turning off the spigot: reducing drug-resistant tuberculosis transmission in resource-limited settings. *International Journal of Tuberculosis and Lung Disease*, 14(10): 1233-1243

Nigussie, D., Davey, G., Legesse, B., Fekadu, A. and Makonnen, E., (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary Medicine and Therapies,* 1(21)

Olajuyigbe, O.O., Afolayan, A.J., (2012). In vitro ethnotherapeutic potential of the acetone extract of the bark of *Ziziphus mucronata Willd. subsp. mucronata Willd*.: Antimicrobial and toxicity evaluations. *African Journal of Biotechnology,* 11: 16783

Parson, E.A., V.R. Burkett, K. Fisher-Vanden, D.W. Keith, L.O. Mearns, H.M. Pitcher, C.E. Rosenzweig, and M.D. Webster, (2007). Global-Change Scenarios: Their Development and Use. In Sub-report 2.1B of Synthesis and Assessment Product 2.1 by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. *Department of Energy, Office of Biological & Environmental Research.*

Patel, K. and Patel, D., (2019). The beneficial role of rutin, a naturally occurring flavonoid in health promotion and disease prevention: A systemic review and update. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, 457-479

Priya, M.R. and Priya, R.I., (2020). Antiproliferative effects on tumor cells of the synthesized gold nanoparticles against Hep2 liver cancer cell line. *Egyptian Liver Journal*, 10(15)

Rajan, A., Vilas, V. and Philip, D., (2015). Studies on catalytic, antioxidant, antibacterial and anticancer activities of biogenic gold nanoparticles. *Journal of Molecular Liquids*, 212: 331-339

Ramabulana, A.T., Petras, D., Madala, N.E. and Tugizimana, F., (2021). Metabolomics and molecular networking to characterize the chemical space of four *Mormodica* plant species. *Metabolites*, 11(11): 763

Reynolds, Y., (2001). *Peltophorum africanum Sond*. Kirstenbosch NBG

Rho, H.S., Ghimeray, A.K., Yoo, D.S., Ahn, S.M., Kwon, S.S., Lee, K.H., Cho, D.H. and Cho, J.Y., (2011). Kaempferol and kaempferol rhamnosides with depigmenting and anti-inflammatory properties. *Molecules*, 16: 3338-3344

Richter, E., Weizenegger, M., Rüsch-gerdes, S. and Niemann, S., (2003). Evaluation of genotype MTBC assay for differentiation of clinical *Mycobacterium tuberculosis complex* isolates. *Journal of Clinical Microbiology*, 41(6): 2672-2675

Rohini, S., Vishnupriya, P., Rakshagan, V. and Jain, A.R., (2018). Protective effects of theaflavin. *Drug Invent Today*, 10(10): 2097-2101

Santha, T., Garg, R. and Frieden, T. R., (2002). Risk factors associated with default, failure and death among tuberculosis patients treated in a DOTS programme in

Tiruvallur District, South India, 2000. *International Journal of Tuberculosis and Lung Disease*, 6(9): 780-788

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. and Latha, L., (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines,* 8(1).

Sathiyavimal, S., Vasantharaj, S. and Bharathi, D., (2018). Biogenesis of copper oxide nanoparticles (CuONPs) using *Sida acuta* and their incorporation over cotton fabrics to prevent the pathogenicity of Gram-negative and Gram-positive bacteria. *Journal of Photochemical Photobiology B,* 188: 126-134

Semenya, S.S. and Maroyi, A., (2019). Ethnobotanical survey of plants used by Bapedi traditional healers and its opportunistic infections in the Limpopo Province, South Africa. *South African Journal of Botany*, 122(2019): 401-421

Shankar, P.D., Shobana, S. and Karuppusamy, I., (2016). A review on the biosynthesis of metallic nanoparticles (gold and silver) using bio-components of microalgae: formation mechanism and applications. *Enzyme Microbiology Technology,* 2016(95): 28-44

Shedbalkar, U.U., Singh, R., Wadhwani, S.A. and Chopade, B.A., (2014). Microbial synthesis of gold nanoparticles: current status and future prospects. *Advanced Colloid Interface Science*, 209: 40-48

Shukla, R., Pandey, V., Vadnere, G.P. and Lodhi, S., (2019). Role of flavonoids in management of anti-inflammatory disorders. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, 293-322

Singh, R., Kumar, V., Bharate, S. S., and Vishwakarma, R. A., (2017). Synthesis, pH dependent, plasma and enzymatic stability of Bergenin prodrugs for potential use against rheumatoid arthritis. *Bioorganic & Medical Chemistry*, 25(20); 5513-5521

Smith, I., (2003). *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clinical Microbiology Review*, 16(3): 463-496

Speshock, J.L., Murdocks, R.C., Braydich-Stolle, L.K., Schrand, A.M. and Hussain, S.M., (2010). Interaction of silver nanoparticles with *Tacaribe* virus. *Journal of Nanobiotechnology*, 8(19): 1-9

Street, R.A. and Prinsloo, G., (2012). Commercially important medicinal plants of South Africa: A review. *Bioactive Natural Products,* 2013: 1-16

Tăbăran, A.F., Matea, C.T., Mocan, T., Tăbăran, A., Mihaiu, M., Iancu, C. and Mocan, L., (2020). Silver nanoparticles for the therapy of tuberculosis. *International Journal of Nanomedicine*, 2020(15): 2231-2258

Tamilarasi, P. and Meena, P., (2020). Green synthesis of silver nanoparticles (Ag NPs) using *Gomphrena globose* (Globe amaranth) leaf extract and their characterization. *Materials Today: Proceedings*, 33: 2209-2216

Tang, J., Diao, P., Shu, X., Li, L. and Xiong, L., (2019). Quercetin and quercitrin attenuates the inflammatory response and oxidative stress in LPS-induced RAW 264.7 cells: In vitro assessment and a theoretical model. *BioMed Research Journal*, 2019: 1-8

Thakkar, K.N., Mhatre, S.S. and Parikh, R.Y., (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine*, 6: 257-262

Thoen, C.O., Steele, J.H. and Kaneene, J.B., (2014). Zoonotic Tuberculosis: Mycobacterium Bovis and Other Pathogenic Mycobacteria. *John Wiley & Sons*

Tomaino, A., Matorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C. and Saija, A., (2010). Antioxidant activity and phenolic profile of Pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie,* 92(9): 1115-1122

Truong, D., Nguyen, D., Ta, N., Bui, A., Do, T. and Nguyen, H., (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxiflolia*. *Journal of Food Quality*, 2019: 1-9

Van Wyk, B.E. and Gericke, N., (2003). People's plants. A guide to useful plants of Southern Africa. *Briza Publications*, Pretoria, 352

Veerapur, V. P., Prabhakar, K. R., Thippeswamy, B. S., Bansal, P., Srinivasan, K. K., and Unnikrishnan, M. K., (2012). Antidiabetic effect of *Ficus racemosa* Linn. stem bark in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats: a mechanistic study. *Food Chemistry*, 132; 186-193

Vijaya Kumar, V.P., Kala, S.M.J. and Prakash, K.S., (2019). Green synthesis of gold nanoparticles using *Croton caudatus geisel* leaf extracts and their biological studies. *Material Letter*, 236: 19-22

Wang, K., Li, Y. F., Lv, Q., Li, X. M., Dai, Y., and Wei, Z. F., (2017). Bergenin, acting as an agonist of PPAR gamma, ameliorates experimental colitis in mice through improving expression of SIRT1, and therefore inhibiting NF-kappa B-mediated macrophage activation. *Frontiers of Pharmacology*, 8: 981

World Health Organization. Global Tuberculosis Report, (2019). Geneva: *World Health Organization*; 2019. License: CCBY-NC-SA3.olGO

World Health Organization, (2011a). Guidelines for intensified tuberculosis case finding and isoniazid preventive therapy for people living with HIV in resource-constrained setting. World Health Organization, 2011, ISBN 978 92 4 150070 8

World Health Organization (2011b). Tuberculosis MDR-TB & XDR-TB 2011 progress report, Who Report: Towards universal access to diagnosis and treatment of MDR-TB &XDR-TB by 2015, 23 March 2011, p.2, ISBN 978 92 4 150133 0. 2011b

World Health Organization. Global Tuberculosis Report (2020a). [Internet]. WHO. World Health Organization; [cited 2021 October 18]. Available from: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf

Xu, Q., Fu, Q., Li, Z., Liu, H., Wang, Y., Lin, X., He, R., Zhang, X., Ju, Z., Campisi, J., Kirkland, J.L. and Sun, Y., (2021). The flavonoid procyanidin C1 has senotherapeutic activity and increases lifespan in mice. *Nature Metabolism*, 3(2021): 1706-1726

Zahoor, A., Sharma, S. and Khuller, G.K., (2005). Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. *International Journal of Antimicrobial Agents*, 26(4): 298-303

Caminero, 2005

Eloff, 1998

Farkas et al., 2018

Hussain et al., 2013;

Hussein et al, 2020

Lawn & Wilkison, 2006.

Mabasa et al. (2021

Mazimba, 2014

Sameera and Mandakini, 2015;

WHO,2013