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Green synthesis and characterization of silver nanoparticles from the cocktail of *Capparis sepiaria-Tabernaemontana elegans* extracts and assessment of their biological effects *in vitro*.

by

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ABSTRACT

Green synthesis and characterization of silver nanoparticles from the cocktail of *Capparis sepiaria-Tabernaemontana elegans* extracts and assessment of their biological effects *in vitro*.

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Antimicrobial Resistance (AMR) is a global crisis that develops when bacteria, viruses, fungi, and parasites adapt and multiply in the presence of drugs that once negatively affected them. AMR infections are commonly caused by the overuse and misuse of antimicrobial drugs, thus leading to severe illnesses, longer hospitalization, increased healthcare expenses, treatment ineffectiveness, and increased mortality. The increasing incidence of AMR poses a serious threat to public health. As a result, alternative strategies that are both effective against AMR pathogens and eco-friendly are urgently needed. Green nanotechnology, in particular the use of silver nanoparticles (AgNPs), has been used as a solution in a wide range of applications, including antibacterial, antiviral, and antifungal therapies. Over the last decades, several medicinal plant extracts have been used to synthesize AgNPs; however, combining extracts from two medicinal plants to synthesize AgNPs with enhanced properties has received less attention. Therefore, this study reports on the green synthesis of AgNPs using a cocktail of *Capparis sepiaria–Tabernaemontana elegans* (CsTe) aqueous extracts as a reducing, stabilizing, and capping agent, and evaluation of their antimicrobial activities.

During the synthesis of CsTe-AgNPs, various parameters such as pH, temperature, extracts and silver concentrations, reaction ratio, and time of synthesis were optimized. The CsTe-AgNPs were successfully synthesized at two pH (6 and 11) and characterized using Ultraviolet-visible spectroscopy (UV-Vis), Dynamic Light Scattering (DLS), High-Resolution Transmission Electron Microscopy (HR-TEM), Fourier Transform Infrared (FT-IR) spectroscopy. The antimicrobial activity of CsTe-AgNPs was investigated *in vitro* using agar well diffusion and microdilution assays. Then later was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration

(MBC/MFC). The antioxidant properties of CsTe-AgNPs were evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The cytotoxicity of CsTe-AgNPs against normal skin cell line (KMST-6) derived from fibroblast cell type was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The CsTe-AgNPs had a surface plasmon resonance (SPR) peak at 400 and 410 nm for pH 6 and pH 11, respectively. The DLS and HR-TEM data displayed monodispersed and spherical AgNPs, with average hydrodynamic sizes of 23 ± 12.26 and 138 ± 2.086 nm, and core sizes of 14 ± 2.953 and 7 ± 3.849 nm for pH 6 and pH 11, respectively. The PDI of 0.271 ± 0.049 and 0.322 ± 0.043 and ζ-potential of -20 ± 0.583 and -24.9 ± 0.705 mV were for CsTe-AgNPs at pH 6 and pH 11. The FTIR analysis which was performed to identify the functional groups that are involved in the stabilization and/or reduction of the CsTe-AgNPs, revealed a shift in peaks of biomolecules present in the plant extracts responsible for the reduction of Ag salt to form CsTe-AgNPs. The agar well diffusion and microdilution assay displayed CsTe-AgNPs to have potent antimicrobial activity, with a MIC of 12.5 ± 0 µg/mL against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and slightly higher MIC for *Candida albicans* of 25 ± 0 µg/mL. The cytotoxicity of CsTe-AgNPs and extracts were evaluated on KMST-6 normal skin cell line, and it was observed that CsTe-AgNPs were slightly toxic to the normal skin at all concentrations used (0.78 - 50 µg/mL), whereas extracts displayed dose-dependent cytotoxic activity against the normal skin cell line. Therefore, this study demonstrated the effectiveness of using a plant cocktail to synthesize AgNPs with enhanced antimicrobial activity and could serve as a promising strategy to combat AMR.

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KEYWORDS

Nanotechnology

Silver nanoparticles

Green synthesis

Capparis sepiaria

Tabernaemontana elegans

Plant-mediated cocktail.

Antimicrobial resistance

DECLARATION

I declare that **"*Green synthesis and characterization of silver nanoparticles from the cocktail of Capparis sepiaria-Tabernaemontana elegans extracts and assessment of their biological effects in vitro*"** is my own work, that it has not been submitted for any degree or examination in any other university and all the sources I have used or quoted have been indicated & acknowledged by complete references.

Full name: Cate Malope Mashilo **Date:** January 2024

Signed: 

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I would like to dedicate this thesis to the **Almighty God**. Drawing inspiration from Philippians 4:13 **"*I can do all things through Christ who strengthens me*."** I acknowledge the divine support and guidance that has empowered me through this academic journey.

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continue to rest in peace, Daddy.

Noko ya bo Mmaseala!

LIST OF ABBREVIATIONS

°C	Degrees Celsius
%	Percentage
µL	Microliter
µg	Microgram
ζ-potential	Zeta potential
Ag	Silver
Ag^{+}	Silver ion
Ag^{0}	Silver
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
AIDS	Acquired Immuno-deficiency syndrome
Au	Gold
AuNPs	Gold nanoparticles
AMR	Antimicrobial resistance

C. albicans	*Candida albicans*
Co	Cobalt
Cs	*Capparis sepiaria*
CsTe-AgNPs	*Capparis sepiaria-Tabernaemontana elegans* silver nanoparticles
CuO	Copper oxide
ddH_20	Deionised water
DLS	Dynamic Light Scattering
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco Modified Eagle's Medium
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
FBS	Fetal Bovine Serum
FT-IR	Fourier Transmittance Infrared
GO	Graphene Oxide
HIV	Human immunodeficiency virus

HR-TEM	High Resolution Transmission Electron Microscope
IR	Infrared
KBr	Potassium Bromide
K. pneumoniae	*Klebsiella pneumoniae*
KMST-6	Kawasaki Medical School Transformed-6
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MDR	Multidrug resistance
MHA	Müller-Hilton agar
MHB	Müller-Hilton broth
MRI	Magnetic Resonance Imaging
min (s)	Minute (s)
mL	Millilitre
mm	Millimetre
mM	Millimolar

MRSA	Methicillin-resistance staphylococcus aureus
MTT	3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium bromide
NA	Nutrient Agar
NPs	Nanoparticles
PDA	Potato-Dexton agar
PDB	Potato-Dexton broth
PDI	Polydispersity Index
P. aeruginosa	*Pseudomonas aeruginosa*
ROS	Reactive Oxygen Species
S. aureus	*Staphylococcus aureus*
SPR	Surface plasmon resonance
Te	*Tabernaemontana elegans*
TEM	Transmission Electron Microscope
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible spectroscopy
ZnO	Zinc Oxide

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CHAPTER 1

1.1 INTRODUCTION

Antimicrobial resistance (AMR) is one of the major public health threats of the twenty-first century, threatening the effective prevention and treatment of common infectious diseases (Prestinaci *et al.,* 2015, Franklin *et al.,* 2021, Bloom and Cadarette, 2019). The causes of AMR includes overuse and misuse of antimicrobial drugs (Shembo *et al*., 2022, Guo *et al.,* 2019), insufficient research and development of novel antimicrobial agents (Jabbari Shiadeh *et al.,* 2019), as well as inadequate infection prevention and control strategies (Matsui *et al.,* 2023). AMR infections lead to severe illnesses and longer hospitalization, increased healthcare expenses, treatment ineffectiveness and increased mortality (Dadgostar, 2019, Prestinaci *et al.,* 2015, Shrestha *et al.,* 2018, Gupta and Birdi, 2017, Hamers *et al.,* 2021).

Various measures have been taken to address this issue; however, the trend of global AMR shows no signs of slowing down (Dadgostar, 2019). Thus, emphasizing an urgent need to find novel molecules with new spectrum of bioactivity on alternative drug targets. Recently, there has been a surge of interest in the use of nanotechnology to synthesize particles at a nanometer scale. Nanotechnology is the field of science and engineering that design, manipulates and produce materials on the nanoscale, typically between 1 and 100 nm (Gour and Jain, 2019). At this scale, materials often show distinctive physical and chemical qualities as compared to their bulk counterparts and that can be used for a variety of purposes, such as antibacterial and antiseptic agents (Simon *et al.,* 2021).

Metallic nanoparticles (MNPs), such as silver nanoparticles (AgNPs) are of particular interest in combating AMR, as they exhibit remarkable antimicrobial activity against various bacteria, fungi, and virus strains due to their incredibly small size and large surface area (Ahmad *et al.,* 2020, Habeeb Rahuman *et al.,* 2022). These properties make them attractive candidates for the development of antimicrobial agents. AgNPs have been widely used in a variety of medical applications as therapeutic agents. One advantage of using AgNPs is their less toxic nature to humans and highly toxic to microorganisms when used at therapeutic concentrations (Keshari *et al.,* 2020, Shafaghat, 2015, Abdel-Aziz *et al.*, 2014). As a result, AgNPs have been extensively used as therapeutic agent and has become a paradigm of success that may aid in the fight against AMR infections (Dos Santos *et al.,* 2014).

Various techniques have been used in the synthesis of AgNPs, including physical, chemical, and green synthesis (Tien *et al.,* 2008, Mallick *et al.,* 2004, Abdelghany *et al.,* 2018). However, the toxic nature of the chemical and physical methods constrains their use in biomedical applications (Simon *et al.,* 2021). Green synthesis is a more appealing method that is environmentally friendly, does not require the use of toxic chemicals, high pressure, energy, or temperatures, as compared to chemical and physical method (Santhoshkumar *et al.,* 2011). The use of plants, fungi, and bacteria to synthesise nanoparticles (NPs) is a rapidly evolving and growing technique in the field of nanotechnology (Pantidos and Horsfall, 2014, Ahmad *et al.,* 2019, Mustapha *et al.,* 2022). The use of plants extracts is thought to be safe and less-toxic, particularly when contrasted with specific chemical processes or microbial cultures. Furthermore, the bioactive compounds in plants are used as reducing and stabilizing agents.

Over the years several plants have been successfully used to synthesize AgNPs; including, *Cotyledon orbiculata* (Tyavambiza *et al.,* 2021), *Anthemis atropatana* (Dehghanizade *et al*., 2018), *Salvia spinosa* (Pirtarighat *et al.,* 2019), *Handelia trichophylla* (Yazdi *et al.,* 2019), *Artemisia oliveriana* (Fard *et al.,* 2018), *Cuminum cyminum* (Karamian and Kamalnejad, 2019), *Caesalpinia pulcherrima* (Moteriya and Chanda, 2018), *Dillenia indico* (Nayak *et al*., 2020), *Duchesnea indica (Ilahi et al.,* 2021), *Anthemis pseudocotula Boiss* (Ajlouni *et al.,* 2022), *Juniperus procera* (Khan *et al.,* 2022), and their biological activities have been well documented. The use of plant cocktails to synthesize AgNPs have also been documented (Adedeji *et al.,* 2022), however it has received scant attention.

In this study, two indigenous South African plants, *Capparis sepiaria* (Cs) and *Tabernaemontana elegans* (Te), were combined and used to synthesise AgNPs. *C. sepiaria* is a hedge plant with numerous branches, and slender prickly shrubs. The roots, flowers, and leaves are used to treat toxemia, cough, muscular diseases, tumours, inflammation, and skin conditions (Selvamani *et al.,* 2008). *T. elegans* , is a small tree that is widely distributed in the coastal scrub forest and low-lying evergreen river fringes (Pallant *et al.,* 2012). According to reports, the extracts is usually used to wash wounds and as a beverage to treat pulmonary conditions and chest pain. The fruits, seeds and root bark are used to treat cancer, heart disease and a root decoction is said to have aphrodisiac properties (Mansoor *et al.,* 2013, Pallant *et al.,* 2012). The antimicrobial activity of the two plants have been investigated; however, no prior study has adequately considered the possibility of combining *C. sepiaria* and *T. elegans*. Therefore, the current study aims to synthesize, characterise, and evaluate the biological activity of the AgNPs synthesized from the cocktail of the two-plant extracts.

1.1.1 Hypothesis

- The green synthesized AgNPs from cocktail of *C. sepiaria-T. elegans* (CsTe) extracts will have enhanced biological activity as compared to plant extracts alone.

1.1.2 Aim

- To synthesize and characterise *C. sepiaria-T. elegans* AgNPs (CsTe-AgNPs) and assess their antimicrobial, antioxidant, and cytotoxic effects *in vitro.*

1.1.3 Objectives

- To synthesize and characterize CsTe-AgNPs
- To investigate the antibacterial activity of CsTe-AgNPs
- To investigate the antifungal activity of CsTe-AgNPs
- To investigate the antioxidant capacity of CsTe-AgNPs
- To investigate the cytotoxicity effects of CsTe-AgNPs

1.1.4 Research question

- Will the cocktail of CsTe water extracts successfully synthesize AgNPs?
- Will the synthesized AgNPs have enhanced biological activity as compared to the extracts?

1.1.5 Significance of the study

The rise of AMR in pathogenic microorganisms has become a significant public health issue, as it restricts the efficacy of existing antimicrobial agents. This phenomenal leads to increased rates of morbidity and mortality, as infections caused by resistant pathogens are more difficult to treat. Moreover, AMR imposes a significant economic burden. Therefore, necessitating the development of new alternative strategies to combat AMR. Green nanotechnology, in particular the use of medicinal plants to synthesize AgNPs comes as a cost-effective and less toxic solution. *C. sepiaria* and *T. elegans* have been identified for their antimicrobial properties against various pathogens. The synergistic effects of these plants extract in combination with the unique properties of AgNPs holds a promise in combating AMR. AgNPs have distinctive characteristics that make them exceptionally efficient in inhibiting various pathogens including Gram-positive and Gram-negative bacterium, viruses and fungal strains. In addition, AgNPs exhibit the ability to address the problem of resistance development, which is common with

conventional antimicrobial agents. The AgNPs mechanism of action includes targeting multiple sites within microorganisms, thus reducing the likelihood of microorganisms gaining resistance.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Introduction to nanotechnology and nanoparticles

Looking at the power of human imagination and scientific innovation, the study of nanotechnology breakthroughs stands out. Nanotechnology is a fascinating, and interdisciplinary field of research that has expanded very rapidly over the past few decades (Umoren *et al*., 2014). Nanotechnology is the study that manipulates, designs, examines, and manages materials on the nanoscale, typically between 1 and 100 nm (Gour and Jain, 2019, Srilatha, 2011). The prefix "nano" is derived from the Greek word "nanos" which means dwarf (Joudeh and Linke, 2022, Ranjan *et al.*, 2014). A nanometer (nm) is equal to one billionth of a metre, or roughly one hundred thousandth of the width of a human hair (Ranjan *et al*., 2014). By using materials at a nanoscale enables scientists to investigate and benefit from unique properties and phenomena that they possess.

Nanoparticles (NPs) are incredibly tiny particles that can be divided into various categories namely; zero-dimension (0-D), one-dimension (1-D), two-dimension (2-D), and three-dimensions (3-D) of 100 nanometers or less (Revell, 2006). Each category possesses distinct, unique properties and applications. For example, zero-dimension, such as nano dots; their length, width, and height are all fixed at a single point. One-dimension systems, such as graphene, can only have one parameter. Two-dimensional materials, such as carbon nanotubes (CNTs), have length and width. Lastly, three-dimensional particles, like gold nanoparticles (AuNPs), have all the dimensions-length, width, and height (Ealia and Saravanakumar, 2017). In addition, NPs can also be categorised based on their chemical, physical, and compositional properties: organic, inorganic, and carbon-based NPs (Choudhary *et al.,* 2023). These categories of NPs yield unique physicochemical properties such as their small sizes, large surface area to mass ratios, high electrothermal conduction capacities, reactive surface groups, and diverse chemical compositions which make them have increased biological activities (Yu *et al.,* 2016).

In recent years nanotechnology research has emerged into a novel, multidisciplinary field bringing together expertise from various fields including physics, biology, metal science, chemistry and engineering amongst others. It presents brand-new answers to difficult problems (Saliminasab and Ghahramani, 2022). In the era of nanotechnology, the demand for nanomaterials research is increasing

daily, due to the fact that nanoscale metals exhibit unique and superior properties when compared to bulk metals (Shafaghat, 2015).

The potential benefits of nanotechnology are many and diverse. Nanotechnology helps develop innovative methods to produce new products, and reformulate new materials and chemicals with improved performance, reduced energy consumption, reduced environmental harm and material use (Ali Mansoori *et al.,* 2008). This technology has the potential to change the world perspectives and expectations as well as give the ability to solve problems on a global scale using less energy and resources, aligning with sustainability and resource conservation principle.

Research on the use of NPs in various fields is essential for promoting creativity, tackling worldwide issues, enhancing effectiveness, and building a more knowledgeable and sustainable world (El-Sadik *et al.,* 2010, Mustafa *et al.,* 2021). Nanotechnology has significantly influenced various fields including, agricultural, environmental, food industry, science and biomedical fields amongst others (**Figure 2.1**) (Chiozzi and Rossi, 2020). For example, in the agriculture sector NPs enhance crop yield, nutrient delivery, and food packaging (Panda *et al.,* 2020, Alvarado *et al.,* 2019). In the biomedical applications, NPs are used as drug delivery, gene therapy, cancer detection, diagnosis, drug transport, biomarker mapping, targeted therapy, and molecular imaging (Jin *et al.,* 2020b, Hulla *et al.,* 2015). These represents few of the promising outcomes that have come from the use of nanotechnology in cancer diagnosis and treatment.

2.2 Significance and application of NPs in various fields

2.2.1 Agriculture

The agricultural sector serves as the backbone of numerous developing countries, and plays an important role by ensuring food security (Habeeb *et al*., 2022, Trevor and Kwenye, 2018). Numerous issues and obstacles, such as climate change, depletion of natural resources, environmental pollution, and soil degradation are often encountered in the agricultural field (Omara *et al.,* 2019). Of particular concern is the impact of pathogenic entities that causes plant diseases, thus posing a significant challenge to global food security within the agricultural sector (Kumar *et al.,* 2022). Nanotechnology offers potential for the precise release of agrochemicals and targeted delivery of macromolecules (Nair *et al.,* 2010). This has the potential to enhanced plant disease resistance, improved nutrient utilisation, and increased plant growth. Nanomaterials have different applications within the agricultural domain including, their role as

nano-fertilisers in improving nutrient absorption by plants and the reduction of nutrient loss through run-off, ultimately leading to an enhancement in food production (Choudhary *et al.*, 2023). Furthermore, nanotechnology plays an important role in the development of nano-pesticides that controls pest population (Zhang *et al.,* 2020, Simonin *et al.*, 2018).

According to the study conducted by Fu *et al.,* (2020), nano-fertilizers promote sustainable agriculture by enhancing nutrient uptake and preserving crops (Fu *et al.,* 2020). Their application aligns with sustainable agriculture and reduce ecological impact associated with traditional agrochemicals. Other studies emphasized in incorporating NPs as bactericides or fungicides in disease management strategy (Elmer *et al.*, 2018). This approach aims to improve plant health and provides benefits of disease prevention.

2.2.2 Food industry

The utilisation of nanotechnology in food science and microbiology has become increasingly necessary. Nanotechnology is crucial in identifying foodborne pathogens, food packaging, and prolong shelf life of food (Balan and Kadeppagari, 2021, Kourkoutas *et al.,* 2016). Nanotechnology integration enhances the lifespan of various food materials, thereby reducing food spoilage caused by microbial infestation (Pradhan *et al.,* 2015). Nanotechnology facilitates the development of new technologies such as antimicrobial agents, high-performance plastics with exceptional barrier properties, and detection techniques for identifying contaminants in packaging procedures (Chellaram *et al.,* 2014). In food preservation, nanotechnology is applied by careful preparing and handling of food to prevent the loss of its natural characteristics and nutrients (Hamad *et al.*, 2018).

Moreover, nanotechnology is used in the field of food packaging. The nano-food packaging materials have several functions, including improving food safety, notifying consumers about possible contamination or spoilage, repairing packaging tears, and releasing preservatives to extend the shelf life of the food inside (Adeyeye, 2019, Han *et al.,* 2011, Rahmati *et al.,* 2020). Nanotechnology has been identified as a transformative force in the food industry due to its wide range of applications, as emphasised by Ashraf *et al.* (2021). It does not only tackle immediate issues concerning food safety and preservation, but also enhances the overall sustainability of food systems.

2.2.3 Cancer therapy

The application of nanotechnology in cancer therapy, have received considerable interest, due to their unique properties that makes them well-suited for specific delivery of drugs, imaging agents, and various therapeutic agents to cancerous cells (Nagachinta *et al.,* 2020, Peng *et al.,* 2023, Gou, 2013). Nanotechnology enabled the development of nanoscale devices such as tumour-specific ligands, antibodies, anticancer medications, and imaging probes, that can be conjugated with multiple functional molecules for various application (Wang *et al*., 2008). These nanodevices, which are 100 – 1,000 times smaller than cancer cells, and may interact with tumour-specific proteins on the surface and within cancer cells (Wang *et al*., 2008, Dhiman *et al.,* 2013).

Conventional chemotherapeutic agents affect both cancerous and normal cells in the body, their distribution throughout the body is non-specific, which limits the dose that can be administered inside the tumour and also leads to treatment that is not as effective because of excessive toxicities (Cho *et al.,* 2008, Li *et al.,* 2022, Yao *et al.,* 2017). Moreover, the majority of current diagnostic methods identify cancer only after metastases, which reduces the anti-cancer medication's efficacy (Singh, 2005). The NPs have the capacity to cross capillaries in cells and tissues due to nano size that can penetrate the cells (Shanaa *et al.,* 2021, Simon *et al.,* 2010, Souri *et al.,* 2022). Nanotechnology makes use of medical devices like imaging probes to differentiate and treat active cancer cells without endangering healthy cells (Singh, 2005). Nanodevices are used as a carriers to deliver drugs to the intended locations, which lowers the harmful side effects brought on by drug entry into other healthy tissues and improves therapeutic efficacy and targeting precision (Zhang, 2021).

2.2.4 Infectious disease control

Infectious disease continues to be one of the most critical global challenge, posing significant medical and technological challenges (Hammond, 2017). Addressing this challenge demands new innovative approach for the treatment, detection, and prevention of the infectious disease. Nanotechnology has emerged as a promising field, providing unique tools and strategies to combat infectious diseases across different levels. NPs possess remarkable properties that makes them highly promising candidates for enhancing diagnostics and the management of infectious diseases. According to Mitchell and Carlson (2018), the unique properties of NPs, such as their small sizes, intrinsic characteristics, and capacity for detailed functioning, makes them highly effective at identifying and selectively engaging with specific diseases.

Due to their special qualities such as small size and high surface area, NPs are used in infectious diseases in a variety of ways to enhance diagnosis, treatment, and prevention (Ansari, 2019). Various types of particles including silver, gold, copper, zinc oxide, titanium oxide particles display antimicrobial activity against various pathogens. Amongst all the particles silver nanoparticles (AgNPs) have broader spectrum against various pathogenic strains, thus making it stands out. AgNPs may be used in wound dressings, coatings, or medical devices to stop bacterial infections and promote healing (Ashmore *et al.,* 2018). According to the study conducted by Ndayishimiye *et al.* (2022), the use of nanomaterial-based approaches holds potential to evade established mechanisms employed by bacteria that have developed resistance to drugs. Moreover, nanomaterials like carbon nanotubes (CNTs) and graphene oxide (GO) have been studied to possess antiviral capabilities (Purwar *et al.,* 2022). By interfering with the structure of viruses and inhibit infection. For the treatment of viral infections like HIV, liposomal NPs contain antiviral medications and can release medications at the desired site and ensure effective drug delivery (Ruiz-Hitzky *et al.,* 2020).

NPs can also be used in the field of medical imaging, particularly in infectious diseases diagnosis. Modern fluorescent NPs are highly sensitive (Qasim *et al.,* 2014). NPs can gather at the infection site and produce finely detailed images that aid in diagnosis (Torrisi and Scolaro, 2017, Schöne *et al.,* 2015). The ability of NPs to produce a strong signal enables the early detection of infections. Magnetic resonance imaging (MRI) can be used to visualise infections by using iron oxide (FeO) NPs as contrast agents (Jain, 2010). In addition to saving the patient, efficient care stops the infections from spreading and reduced side effects are all aided by these NPs.

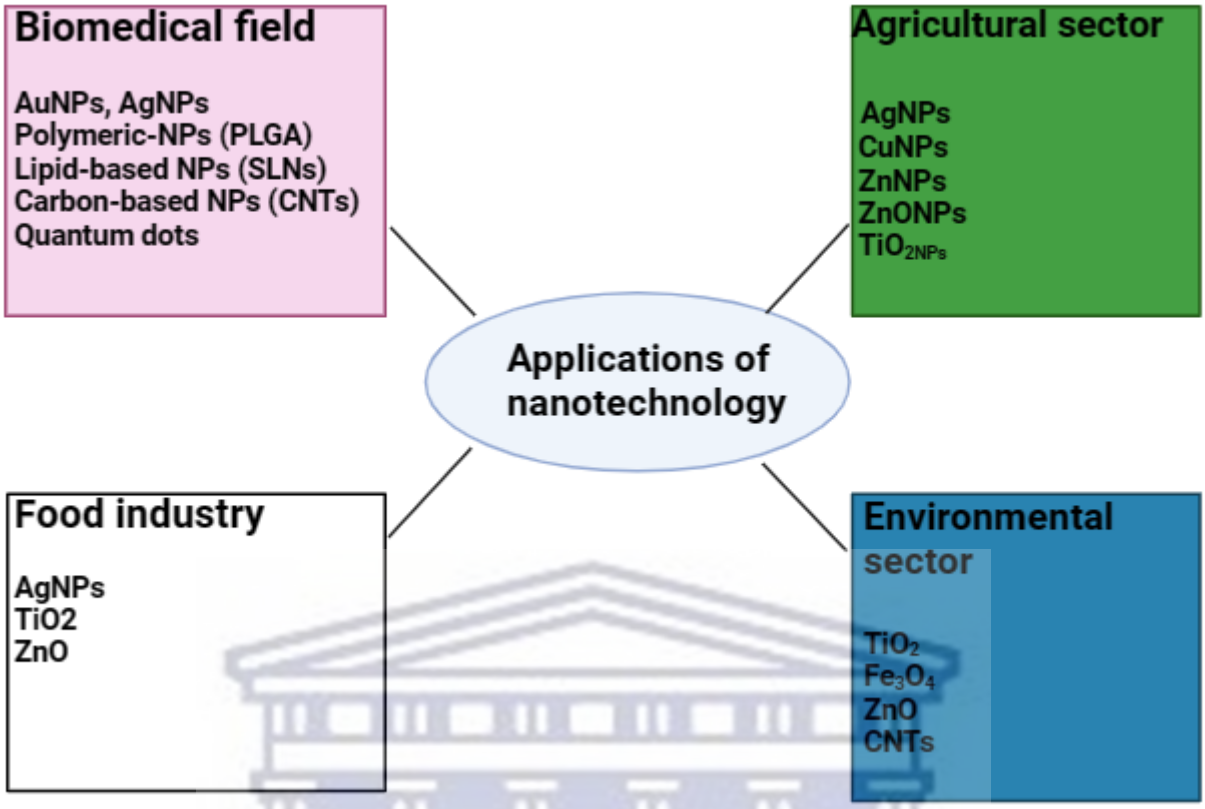


Figure 2.1: Application of nanotechnology in various fields and commonly used particles in each listed field.

2.3 Types of NPs: carbon-based, organic, and inorganic.

Nanoparticles (NPs) are generally categorised into three types: carbon-based, organic, and inorganic. Each class of NPs has unique traits that give them applications that are not found in any other. Due to their unique composition and properties, NPs are adaptable and useful in a variety of fields including drug delivery, electronics, and antimicrobial agents amongst others. Among other qualities, NPs size-to-surface ratio makes them especially well-suited for these wide range of uses. These broad range potential use emphasises how important it is to utilise the unique characteristics that each class of NPs possesses.

2.3.1 Carbon-based nanoparticles

Carbon-based NPs are materials at the nanoscale that consist mainly of carbon atoms. Examples of carbon-based NPs includes, carbon nanotubes (CTNs), fullerenes, graphene, carbon black, and nanofibers (**Figure 2.2**). CNTs represents a class of cylindrical structures composed of carbon atoms arranged in a hexagonal lattice (Dizaj *et al.,* 2015). These CNTs are fascinating materials with unique

properties that make them distinct in terms of their dimensions, configuration, and distinctive physical characteristics, thus making them to be used in various fields (Ealia and Saravanakumar, 2017). CNTs are used in electronics, drug delivery and material science. One unique feature of CNTs is their very tiny macromolecules nature. Despite their small size, they exhibit extraordinary mechanical, electrical and thermal properties (Haleem *et al.,* 2023).

Fullerenes are the hollow cage structure particles with sixty or more carbon atoms (Kourkoutas *et al.,* 2016). These allotropes of carbon, are used in nanotechnology as drug delivery system, and also as antioxidant agents (Kolahalam *et al.,* 2019). Graphene is a single layer of carbon atoms arranged in a hexagonal lattice (Zhu *et al.,* 2012). Graphene materials possess remarkable electronic, mechanical, electrical, and optical characteristics, as well as a chemically tunable surface. These properties make them highly desirable for a variety of applications, including composites, electronics, and nanomedicine (Bussy *et al.,* 2013). Carbon black NPs are made up of very small amorphous carbon particles. Carbon black is common in manufacturing industries and is mostly used to make rubber, printing inks, and paints (Tang *et al*., 2020). Carbon nanofibers are structures with nanoscale diameter used in tissue engineering, and drug delivery.

2.3.2 Organic nanoparticles (NPs)

Organic NPs are small particles made of aggregated molecules or polymer. These NPs are made from organic compounds such as carbon-hydrogen bond. Similar to other particles, organic nanomaterial are a class of molecules that are synthesised into NPs with precise dimensions, typically with a radius of less than 100 nm (Haleem *et al.,* 2023). This category has different types of materials for specific application, including polymeric NPs, liposomes, micelles, ferritin, and dendrimers. Polymeric NPs are produced from their synthetic or natural polymer. One of the advantages of organic NPs is their ability to load materials. They can load molecules either by conjugation on the surface or in the core, or by physical encapsulation, which makes them appealing systems for the delivery of molecules and various biomedical applications (Jeong *et al.,* 2009, Chen *et al.,* 2018). Organic NPs are also used in pharmaceutical formulations, including liposome vectors, polymer-protein, or polymer-drug conjugates. The encapsulation of materials within organic NPs offers relatively simple routes for the encapsulation of materials (Romero and Moya, 2012). Thus, this simplicity contributes to the wide use of organic NPs in various fields.

2.3.3 Inorganic Nanoparticle

Inorganic NPs are those that are synthesised from metals and metal oxide to nanometric sizes using either constructive or destructive methods (Ealia and Saravanakumar, 2017). The commonly used metals for NPs synthesis are aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn). In metal oxide commonly used NPs includes Zinc Oxide (ZnO), Titanium Oxide (TiO) and Iron Oxide (FeO), due to their extensive surface area, controllable structures, varied surface chemistry, and distinctive optical and physical properties. Inorganic NPs have a lot to offer in the biomedical field (Liu *et al.,* 2021).

Inorganic NPs are known for their low toxicity, biocompatibility, and stability. Furthermore, inorganic NPs have ease of synthesis, protection of their therapeutic cargo, and the ability to be modified for conjugation of biomolecules or cell-specific targeting (Zenze *et al*., 2023). Among all types of NPs, metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution (Vanlalveni *et al.,* 2021). Moreover, these have a high surface area and have the good adsorption ability of small molecules (Kolahalam *et al.*, 2019).

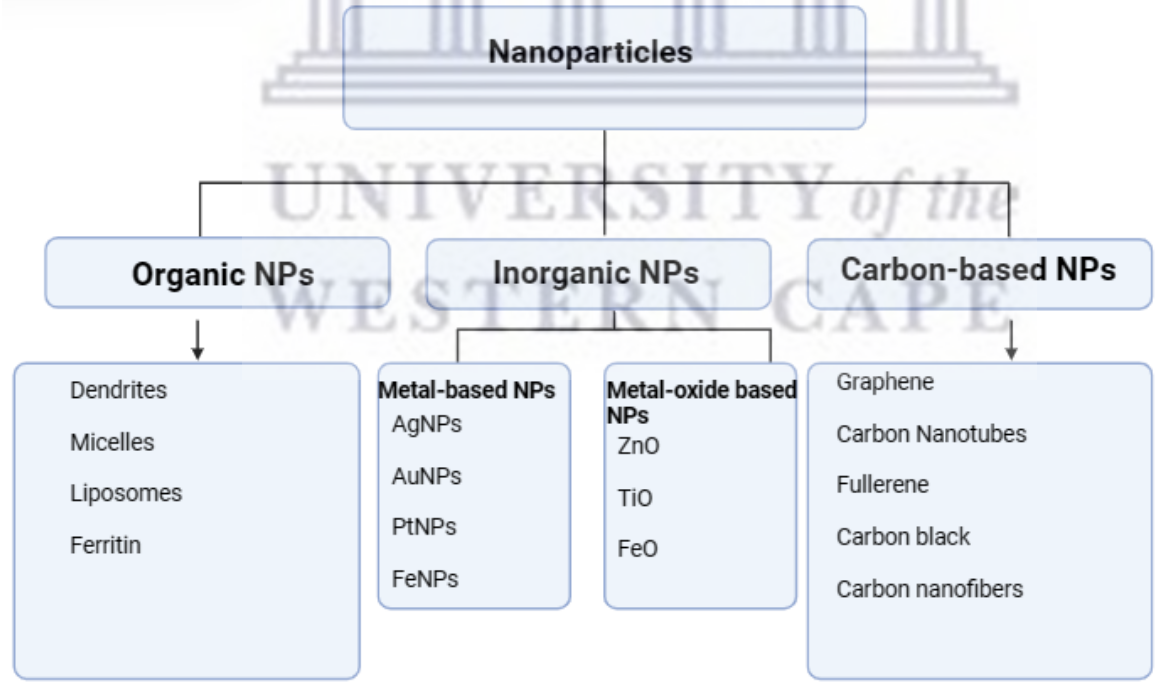


Figure 2.2: Classification of carbon-based, organic and inorganic NPs used in biomedical fields (Adapted from (Raj et al., 2023)).

2.3.3.1 Metallic NPs

Metallic NPs are small with sizes ranging from 1 to 100 nm, usually derived from metal precursor. Numerous types of NPs, including silver (Ag), aluminium (Al), gold (Au), iron (Fe), lead (Pb), cobalt (Co), zinc (Zn), cadmium (Cd), and copper (Cu) have been synthesised. These particles are increasingly being utilised across various disciplines due to their distinctive characteristics that differentiate them completely from those of their original bulk materials. They are extensively used in various practical domains, including targeted drug delivery, magnetic resonance imaging, vaccine administration, biomedicines, drug-gene delivery, cosmetics, optoelectronics, and catalysis (Ilahi *et al.,* 2021). However, AgNPs are the most intriguing particles (Zhang *et al*., 2016). This is mainly due to their broad-spectrum antimicrobial activity (Gopinath *et al*., 2015). AgNPs have been effectively utilized as antibacterial, antifungal, antiviral, anti-inflammatory, anti-angiogenic, and anti-cancer agents (Rai *et al.,* 2021, Zhang *et al.,* 2016).

2.3.4 Silver nanoparticle (AgNPs)

AgNPs have gained significant attention in the search for a new, effective, and affordable antimicrobial agent mainly due to their remarkable and broad-spectrum antimicrobial activity as compared to conventional agents. However, there are some restrictions and considerations associated with their use. It has been reported that AgNPs are less-toxic to humans and highly toxic to microorganisms when used at low concentrations, and their antimicrobial activity are attributed by silver ions (Abdel-Aziz *et al.,* 2014, Keshari *et al.,* 2020, Shafaghat, 2015). The silver ions exhibit toxic effects towards microorganisms, including bacteria, viruses, and fungi (Prabhu and Poulose, 2012). Their toxicity is mainly because silver ions can interfere with vital cellular functions in these microbes and cause disruptions.

Recent research indicates that AgNPs possess enhanced antimicrobial activity due to their increased surface area (Kanipandian *et al.*, 2014). The antimicrobial properties exhibited by AgNPs are associated with their crystallographic surface structure and the increased surface-to-volume ratio that results from their smaller size (Morones *et al.,* 2005, Kora and Arunachalam, 2011). It is likely that the combined effects of the free ions of silver and the activity of NPs work in different ways to produce a potent, broad-spectrum antimicrobial activity (Franci *et al.,* 2015). AgNPs have been utilised effectively as therapeutic agents, serving as a successful model applicable to a various field beyond antimicrobial use. Their versatility extends to wound healing, anti-inflammatory, and even cancer (Pem *et al.,* 2019). In the antimicrobial sector, AgNPs plays a crucial role in applications such as; biosensor materials for their

ability to enhance sensibility and specificity of detection (Ibrahim *et al.,* 2021), water purification by removing contaminants such as heavy metals and organic pollutants in the water source (Ibraheim *et al.,* 2016), cosmetic and personal care products (Szczepańska *et al.,* 2020), medical equipment particularly in the development of diagnostic tools and imaging agents amongst other applications due to their special qualities (Iravani *et al.,* 2014).

2.3.5 Methods of NPs generation

Numerous techniques exist for the synthesis of NPs, broadly classified into two categories: 'Top-down' and 'Bottom-up' approach. In the top-down approach, suitable bulk material is broken down into fine particles using a variety of size reduction techniques (Rafique *et al.,* 2017). Physical techniques such as laser ablation, ball milling, sonication, vaporization, and lithography are common examples of top-down methods (**Figure 2.3**). The bottom-up approach involves the generation of NPs from small units such as molecules and atoms (Lin *et al.,* 2022), or through the self-assembly of atoms into new nuclei, which then grow into a particle with nanoscopic dimensions using a variety of chemical and biological techniques (Jadoun *et al.,* 2021).

Chemical and biological methods are mainly used in Bottom-up approach. Examples of 'bottom-up' approach includes chemical precipitation that makes use of sol-gel synthesis, spray method, condensation, microemulsion and precipitation. Whereas biological approached uses plants and microorganisms as reducing and capping agents. Each technique has its own benefits and drawbacks, with common issues including cost, scalability, particle sizes, and particle size distribution (Natsuki *et al*., 2015). However, amongst all techniques used to synthesize NPs, biological route-based methods are the most attractive due to their less-toxic nature (Simon *et al*., 2021), thus replacing the toxic reducing agents used in chemical synthesis methods and high temperature, and costly equipment in physical methods (Natsuki *et al.,* 2015).

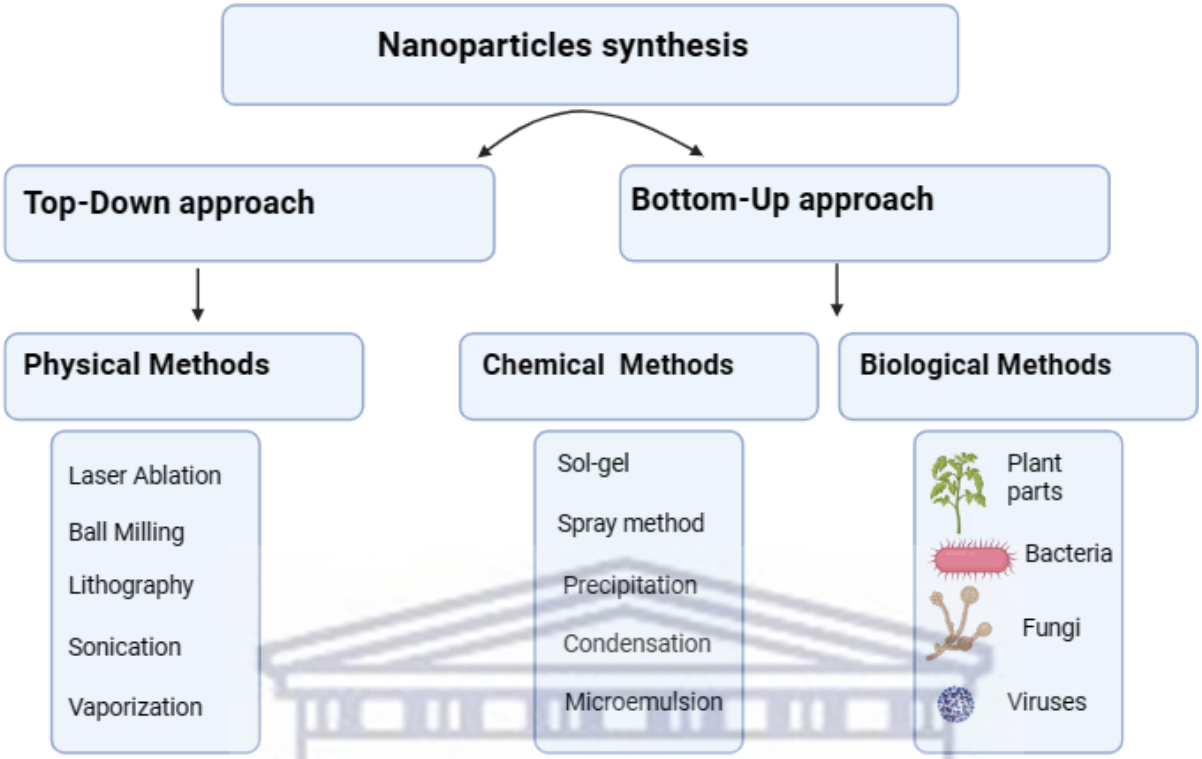


Figure 2.3: Various methods and approaches used in the synthesis of NPs (Adapted from (Rai et al., 2021, Raj et al., 2023)).

2.3.5.1 Chemical and physical methods

Numerous chemical and physical methods are being used these days to produce NPs. However, as mentioned previously these methods have their limitations including high costs, high energy consumption, and utilization of various toxic chemicals during the synthesis. The synthesis of NPs using chemical methods make use of reducing agents, such as sodium borohydride, citrate, or ascorbate (Groiss *et al.,* 2017). These reducing agents have been found to produce toxic byproducts, they involve the use of harmful, hazardous compounds that pose a variety of biological concerns as highlighted by Burange *et al.,* (2021); thus, imposing restrictions on their applicability within the biomedical fields (Al-sherbini *et al.,* 2015).

Physical methods for the synthesis of NPs have their own limitations as well, including high costs, substantial energy and space requirements, and a significant demand for resources (Fard *et al*., 2018). The desiccation condensate technique is most common in physical method and is associated with challenges like consumption of high of amount of energy and contribute to the rise in ambient

temperatures and prolonged duration to achieve thermal stability (Lekha *et al.,* 2021). The tube furnace is a common component in physical methods and contributes to increased temperature and poses environmental concern. Therefore, environmental impacts, cost of synthesis and other drawbacks in physical and chemical methods (Hulikere & Joshi, 2019).

2.3.5.2 Green synthesis of NPs

Recognising the environmental impacts and toxic nature from traditional synthesis methods calls for an alternative environmentally friendly technique. Green nanotechnology involves the utilisation of various biological entities such as plants, fungi, bacteria, and algae for the purpose of synthesising environmentally sustainable NPs (**Figure 2.3**). The utilisation of environmentally friendly, and sustainable approaches offers advantages like reduced costs, enhanced ecological sustainability, and straightforward implementations (Khan *et al.,* 2022). The utilisation of plant extracts is deemed more advantageous compared to alternative methods due to the widespread availability of plants and the potential of their phytochemicals to serve as stabilising and capping agents, facilitating the conversion of silver ions into AgNPs.

2.3.5.3 Bacteria and Fungi-mediated NPs synthesis.

In recent years the use of microorganisms such as bacteria and fungi are gaining interest in the synthesis of NPs. The potential of microorganisms as ecofriendly and cost-effective nano factories to avoid toxic, harsh chemicals and the high energy demand of physiochemical synthesis is significant (Singh *et al.,* 2016). Diverse microorganisms, both prokaryotes and eukaryotes have been used for synthesis of metallic NPs, for example, Ag, Au, Fe, Co and metal oxides such as titanium oxide TiO and ZnO. The synthesis of NPs using microorganisms makes use of intracellular and extracellular synthesis depending on the microorganisms used. The process of using microbes to synthesise NPs extracellularly is essentially determined to be nitrate reductase-mediated synthesis. For example, in the synthesis of NPs the enzyme nitrate reductase secreted by the fungi helps in the bio reduction of metal ions (Hulkoti and Taranath, 2014).

Like it was highlighted above, bacteria and fungi are commonly used to synthesize NPs. However, the use of fungi in the synthesis present certain challenges. The time required for NPs production by fungi ranges from 24 – 120 hrs, which is a major drawback compared to other synthesis methods. In additional the intracellular synthesis of fungi resulted in downstream processing that is difficult and often defeats

the purpose of developing a simple and cheap process (Jeevanandam *et al.,* 2016). In recent years, there has been a growing interest in plant-based nanomaterials due to their significant relevance in various fields, primarily attributed to their unique physicochemical properties (Nayak *et al.,* 2020). Utilising plant extracts is advantageous mainly because it eliminates the need for complex and multi-step procedures such as culturing, microbial isolation, maintenance.

2.3.5.4 Plants-mediated NPs

The utilisation of plants to synthesise NPs is considered superior in comparison to other biological approaches because of their many advantages. This includes their low cultivation costs, short production times, safety, the capacity to increase production volumes (Makarov *et al.,* 2014), and also the abundant availability of plants as a resource (Naganthran *et al.,* 2022). In plant-based NPs, different parts of the plant like leaves, flowers, seeds, bark, stem, roots and the whole plant may be used to synthesize NPs (Vanlalveni *et al.,* 2021). Plant extracts are rich in terms of the variety of biomolecules and metabolites that they contain, including carbohydrates, proteins, vitamins, phenols, flavonoids, and intermediates based on coenzymes that makes them unique and responsible for the formation of NPs (Naseer *et al.,* 2020). All these phytochemicals or secondary metabolites plays a role in the reduction of metal salt to form NPs. The plants materials are more prominent in NP synthesis when compared to microorganisms. This is due to the fact that microbial culture are susceptible to contamination and maintenance difficulties, could infect the experimenter, and has extremely hazardous side effects (Raj *et al.,* 2023).

Over the years various types of plants have been used successfully to synthesize AgNPs; including, *Cotyledon orbiculata* (Tyavambiza *et al.,* 2021), *Anthemis atropatana* (Dehghanizade *et al*., 2018), *Salvia spinosa* (Pirtarighat *et al.,* 2019), *Handelia trichophylla* (Yazdi *et al.,* 2019), *Artemisia oliveriana* (Fard *et al.,* 2018), *Cuminum cyminum* (Karamian and Kamalnejad, 2019), *Caesalpinia pulcherrima* (Moteriya and Chanda, 2018), *Dillenia indico* (Nayak *et al.*, 2020), *Duchesnea indica (Ilahi et al.,* 2021), *Anthemis pseudocotula Boiss* (Ajlouni *et al.,* 2022), *Juniperus procera* (Khan *et al.,* 2022), and the biological activities of their AgNPs have been well documented.

2.3.5.5 Mechanism that mediates the phyto-synthesis of NPs.

Initially in the synthesis of NPs, plant extracts are mixed with metal salt to form metallic NPs (**Figure 2.4**). The phytochemicals in the plant extracts function as reducing and capping agents when the metal salt acts as a precursor to initiate the reaction (Darbar *et al.,* 2019). The process of forming a NPs from

plant extracts is generally divided into three stages: **(1)** activation phase, **(2)** growth phase, and **(3)** termination phase. The activation phase involves the bio reduction of metal salt, in this context the silver salt to form silver ion (**Figure 2.5**). The parameters determining the conditions of the plant leaf extracts (such as types of phytochemicals, phytochemical concentration, metal salt concentration, pH, and temperature) are admitted controlling the rate of NPs formation as well as their yield and stability. The composition of the plant leaf extracts is also an important factor in NPs synthesis, for example different plants comprise varying concentration levels of phytochemicals (Singh *et al.,* 2018). All the listed parameters play an important role in the synthesis of NPs.

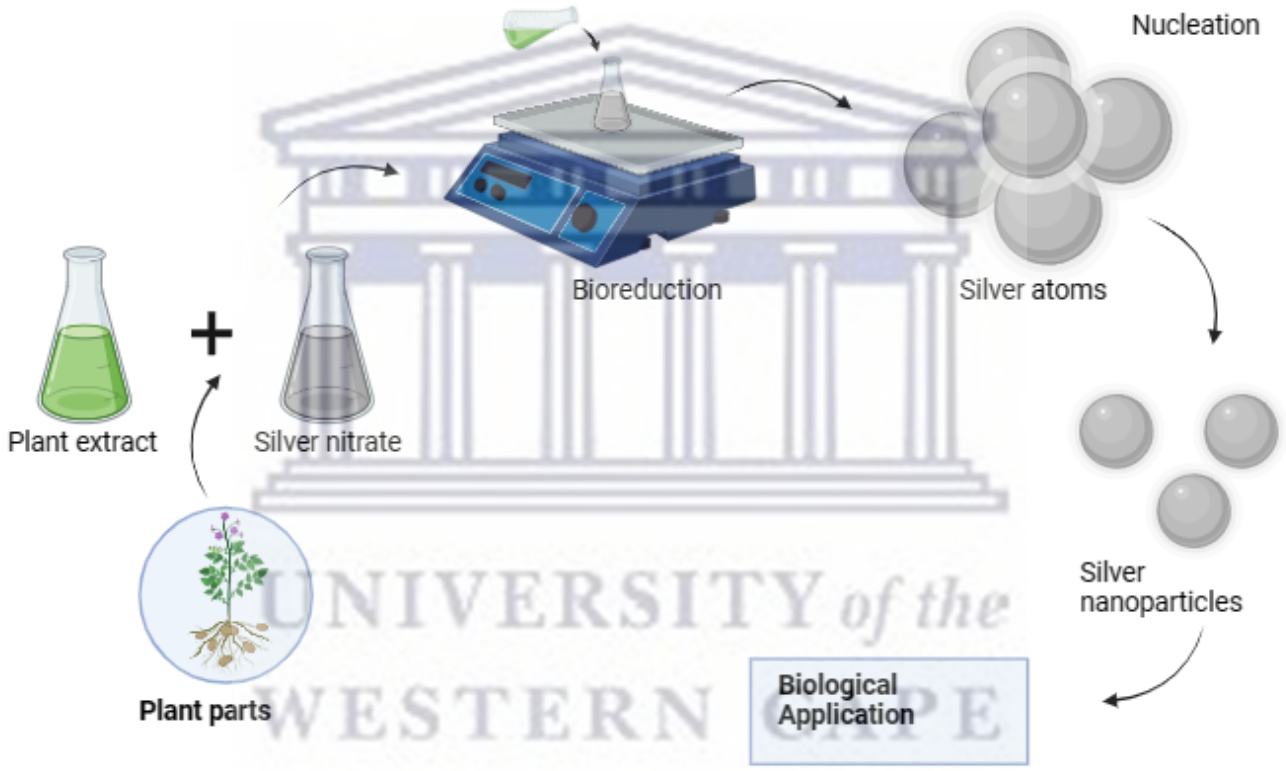


Figure 2.4: Biosynthesis of AgNPs from plants extracts (Adapted from (Raj et al., 2023)

Various studies have indicated that presence of -OH functional groups in plant biomolecules, such as flavonoids, polyphenols, proteins, amino acids, enzymes, steroids, alkaloids, quinones, tannins, saponins, carbohydrates, and vitamins in plant extracts plays an important role (Ratan *et al.,* 2020, Some *et al.,* 2018). This bioactive compound has been linked to the reduction and stabilisation of Ag ions to form NPs. The right mechanism involved in the reduction and stabilization of metal ions has not been clearly understood because biomolecules vary from plant to plant. However, it was discovered that only

flavonoids were involved in the bio reduction and formation of AgNPs (Ratan *et al.,* 2020, Ahmed *et al.,* 2023). The growth phase involves the combination of smaller and larger particles (**Figure 2.5**). This stage involves the overall development and enlargement of NPs. The termination phase is the final stage where in the final shape of the synthesised NPs is defined (Das *et al.,* 2022). According to various studies flavonoids have a variety of functional groups that enable them to undergo structural changes for example tautomeric transformation. When flavonoids undergo tautomeric transformations, the enol-form changes into the keto-form, releasing the reactive hydrogen atom, contributing to the reduction of metal ion, subsequently formation of AgNPs (Singh *et al.,* 2018, Ahmed *et al.,* 2023).

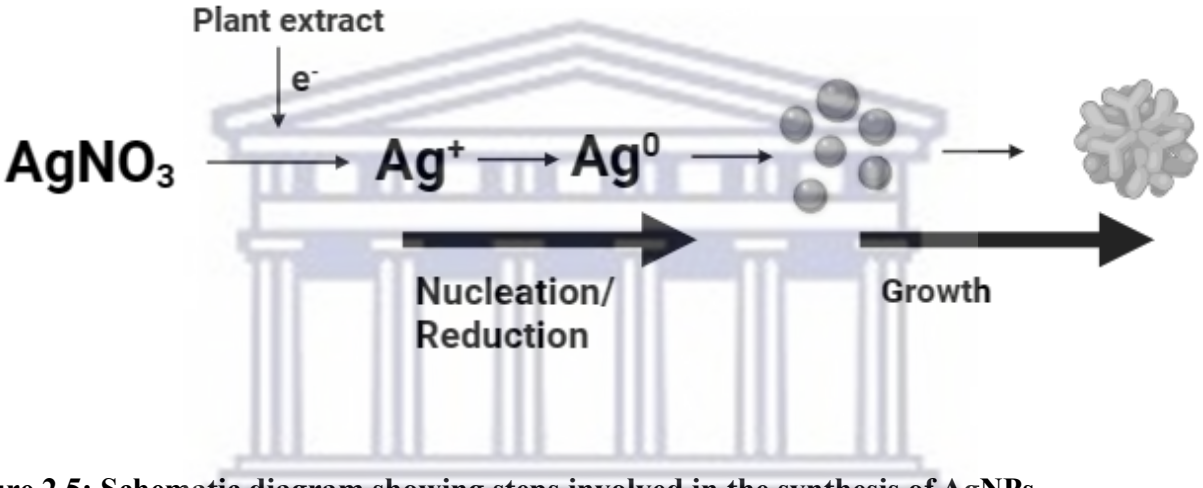


Figure 2.5: Schematic diagram showing steps involved in the synthesis of AgNPs.

2.4 Phyto-medical effects against microbes

Plant metabolites have been used as a source of medicine for a long time, and scientists continues to be very interested in studying them even today. Scientists have found that these secondary metabolites, like alkaloids, flavonoids, terpenoids, and phenolic compounds, have many biological activities including antibacterial properties that make them useful for medicine (Morya, 2021, Lahlou *et al.,* 2022). Some plants have evolved to be able to make compounds which have antimicrobial properties like inhibiting bacteria or fungi from growing (Elsharkawy and El-Khateeb, 2019, Yulianti *et al.,* 2022). These compounds serve as a protective mechanism for the plants, reducing the risk of fungal infections and enhancing overall well-being. Plant metabolites also tend to have fewer side effects than synthetic drugs, which makes them more suitable for long-term use (Kuo *et al.,* 2022).

2.4.1 Medicinal plants properties

Since ancient times, medicinal plants have played a significant role in the realm of medical therapy, serving as a primary source for various remedies and treatments. Medicinal plants have been used as a home remedy from ancient times due to its variety of metabolites and its phytoconstituents (Prabu *et al.,* 2017). These medicinal plants have played an important role in maintaining normal health of human beings and have been a cornerstone in traditional medicine. The phytomedicines derived from herbal plants play a major role in the discovery of new therapeutic agents for drug development (Rai *et al.,* 2020). Low-income individuals in developing nations, such as farmers, residents of small isolated villages, and indigenous communities, use medicinal plants to treat illnesses (Baidya *et al.,* 2022). Scientifically, plants contain bioactive components, such as volatile tannins, oils, alkaloids, flavonoids, and phenols (Adnan *et al*., 2022). These bioactive compounds, have been found to promote a quicker and more efficient healing process for wounds and may play a significant role in the development of new drugs (Yazarlu *et al*., 2021).

Usually, the pharmacological effects of phytochemicals from plants vary and they are diverse. For example, terponoids have antibacterial, anti-inflammatory, anti-cancer, and antiviral effects (Saboon *et al.,* 2019). While flavonoids possess anti-viral, anti-allergic, anti-mutagenic, and anti-inflammatory properties. By mixing these unique properties of plant extracts might open possibilities for developing new therapeutic agents. Extracts from various plants source mixed together to form cocktail might present an innovative strategy. For example, combining an extracts known for wound-healing properties with an extracts known for antimicrobial properties could produce therapeutics optimised for wound dressings (Aremu *et al.,* 2020). This approach might also be used in various fields, including agriculture, water purification, textiles, and cosmetic. Medicinal plants provide wide range of bioactive molecules with potential therapeutic uses.

2.4.2 Medicinal properties of *Capparis sepiaria*

Capparis is a genus of flowering plants that is classified within the *Capparidaceae* family. It is one of the mostly recognised and important ethnomedicinal plants (Boongapim *et al.,* 2009). *Capparis* is the largest genus in the angiosperm family *Capparaceae*, with about 142 taxa. Some taxa, like *C. cartilaginea, C. cleghornii, C. incanescens, C. moonii, C. roxburghii, C. sepiaria*, and *C. spinosa* are part of *Capparis* family and are hard to tell apart based on their morphology, and their ranges partly overlap (Maurya and Choudhary, 2022). These genus *Capparis* is found all over the world, and many of its species have different medicinal uses (Saraswathi *et al.,* 2020). In particular, *Capparis sepiaria* is

common in Pakistan, India, Sri Lanka, and Myanmar (Venugopal *et al.,* 2011). The *C. sepiaria* (*Cs*) commonly known as a hedge plant is a member of the *Capparidaceae* family and is defined by its zigzag stems and narrow, prickly shrubs (Sundaram *et al.,* 2011).

The medicinal systems of Ayurveda and Siddha recognise *C. sepiaria* for its therapeutic properties. It possesses a wide range of pharmacological activities, such as hepato-protective, antidiabetic, anti-tumor, antibacterial, anti-inflammatory, and anti-helminthic effects (Quadri *et al*., 2023). The bark of the *C. sepiaria* root is used in traditional medicine in Cameroon to treat mental and cognitive problems (Yassi *et al.,* 2023). The roots, flowers, and leaves are used to treat toxemia and cough, and it is frequently used to treat muscular diseases, tumours, inflammation, and skin conditions (Selvamani *et al.,* 2008). Studies on *C. sepiaria* extracts and freshly ground fruit were evaluated for antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The findings showed its capacity to inhibit the growth of Gram-negative and Gram-positive bacteria.

Figure 3.1: Photographs of C. sepiaria (Cs) roots

2.4.3 Medicinal properties of *Tabernaemontana elegans*

Tabernaemontana elegans (Te) Stapf, belonging to the Apocynaceae family, is native to tropical and subtropical areas, including Indonesia, Malaysia, and Africa (Paterna *et al.,* 2016). *Tabernaemontana elegans* (*Te*), is a small tree that is widely distributed in the coastal scrub forest and low-lying evergreen river fringes (Pallant *et al.,* 2012). The tree is commonly known as the "toad tree" in English due to the brown, wart-like skin of its fruit. According to reports, South Africans have used *T. elegans* extracts to wash wounds and as a beverage to treat pulmonary conditions and chest pain. The fruits, seeds and root bark are used to treat cancer, heart disease and a root decoction is said to have aphrodisiac properties (Pallant *et al.,* 2012). *T. elegans* root- bark is traditionally believed to possess medicinal properties for treating cancer, according to folklore (Mansoor *et al.,* 2013). This plant species is remarkable for its

production of indole alkaloids that possess distinctive structures and novel bioactivities. *T. elegans* is a notable member, showing the synthesis of unique alkaloid molecules.

Figure 3.2: Photographs of T. elegans (Te) bark.

2.4.4 Properties of CsTe-cocktail

Little research has been done on the use of plant cocktails, which combine several plant extracts for the synthesis process. Cocktail plants of *C. sepiaria* and *T. elegans* are used in this study to synthesize NPs and assess their biological activities *in vitro*. *C. sepiaria* is rich in various bioactive compounds including tannins, flavonoids, saponins and carbohydrates. These bioactive compounds give NPs enhanced antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, *Aspergillus niger, Aspergillus terrius*, and *Aspergillus flavors* and may treat various diseases (Kumar and Prince, 2021). Similarly, *T. elegans* (Te) have bioactive compounds that could contribute to NPs synthesis and exert biological effects. *T. elegans* plants are known for their ability to produce indole alkaloids with unique structures and exceptional bioactivities.

Plant extracts contain a diverse range of phytochemicals that can act as reducing agents aiding in the reduction of metal salts to NPs (Sackey *et al.,* 2021). Through the combined use of various plant extracts, the enhanced bioactivity of NPs can be beneficial in medical contexts, where the possession of antimicrobial, antioxidant, and anti-inflammatory attributes is highly desired (Azizi *et al.,* 2017). By combining plant extracts in NPs synthesis enables enhanced adjustment of the NPs dimensions, morphology, and robustness, making them more appropriate for targeted applications across various domains (Etefa *et al.,* 2023, Gondwal and Joshi nee Pant, 2018). Moreover, by combining extracts with

complementary antimicrobial compounds can result in AgNPs with a broader spectrum of antimicrobial activity, making them more effective against a broader spectrum of pathogens.

2.5 Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is a major public health issue that has been gaining more attention lately. AMR occurs when microorganisms like bacteria, viruses, fungi, and parasites develop and evolve in the presence of medicines that used to kill them (Dadgostar, 2019). AMR is mostly caused by the overuse and improper use of antibiotics in many fields, from healthcare facilities to agriculture (Sakeena *et al*., 2018, Aabenhus *et al.*, 2017). The rise of AMR pathogens increases the vulnerability of susceptible populations such as the elderly, children, and immunocompromised individuals (Tobin and Brenner, 2021, Parveen *et al.,* 2022). The worldwide incidence of resistant infections is increasing, resulting in significant public health consequences and placing a substantial strain on healthcare systems and economies globally (Zharkova *et al.*, 2023).

The global burden of AMR has impact on individuals, communities, and healthcare systems (Campanini-Salinas *et al.,* 2021). For example, in health care system AMR decreases the efficacy of antibiotics, resulting in prolonged and more severe illnesses, elevated healthcare expenses, and increased mortality rates. According to studies that investigated the effects of AMR, predicted that AMR infections will cause nearly 10 million deaths per year by 2050 (Chokshi *et al.*, 2019). Also, an estimated number of 5 million people die from AMR infections, with cases exceeding the total number of deaths from HIV/AIDS and malaria combined (Salam *et al.,* 2023). Moreover, AMR organisms causes more than 2 million infections and are associated with roughly 23 000 deaths each year (Marston *et al.*, 2016).

2.5.1 Mechanism of acquiring AMR

Microorganisms gains resistance to conventional antibiotics using different mechanism. According to Abushaheen *et al*. (2020) and Sefton (2002) resistance is either intrinsic or acquired. Different types of resistance mechanism are possible: **(1)** Bacterial populations acquire resistance genes through mutating, communicating, and sharing resistance genes; **(2)** Resistant bacteria may possess intracellular or extracellular enzymes that degrade, obstruct, or restrict the binding of antibiotics; **(3)** Antimicrobial accessibility within bacterial cells is restricted; **(4)** Active transportation of antimicrobials outside the bacterial cell is facilitated by efflux pumps, which are typically upregulated in resistant bacteria (Ndayishimiye *et al.,* 2022; Reygaert, 2018; Hasan and Al-Harmoosh, 2020). The mechanisms of

resistant by these pathogens include the phenotypic and genetic resistance, as well as conventional antimicrobial therapies. Among the multi-drug-resistant nosocomial organisms are *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

2.5.1.1 *Klebsiella pneumoniae*

Klebsiella pneumoniae, also known as *K. pneumoniae*, is a Gram-negative bacterium capable of causing various infections such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (Cai *et al.,* 2016, Bhardwaj *et al.,* 2020, Min *et al.,* 2019). This bacterium has developed diverse mechanisms to resist the impacts of multiple antibiotics. One of the possible mechanism used by the bacterium includes the production of enzymes that can stop antibiotics from working (Jiang *et al.,* 2014). *K. pneumoniae* produce enzyme ESBL, this enzyme is capable of hydrolysing and deactivating a broad spectrum of beta-lactam antibiotics, including penicillin and cephalosporins thus gaining resistant against them (Ameshe *et al.,* 2022, Akdoğan *et al.,* 2020, Jin *et al.,* 2020a). In addition, *K. pneumoniae* has the potential to generate carbapenemase enzymes, which degrade carbapenem medications and inhibit their efficacy (Karami-Zarandi *et al.,* 2023, Kidd *et al.,* 2017). Another mechanism used by the bacterium involves alterations in the binding sites of antibiotics, which prevent the efficient targeting and inhibition of bacterial growth by antibiotics. This modification of binding sites represents a type of acquired resistance in which K. pneumoniae bacteria acquire the ability to evade the effects of antibiotics through genetic modifications in their DNA (Alrebish *et al.,* 2022). Therefore, these resistance mechanisms contribute to *K. pneumonias* ability to develop multi-antibiotic resistance.

2.5.1.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one example of an opportunistic organism capable of developing resistance to a different type of antimicrobial agents. Similar to other pathogens, this bacterium uses several mechanisms to gain resistance against various microorganisms. These mechanisms used by *P. aeruginosa* includes the formation of a biofilm. Biofilms serve as a protective shield, enhancing the resistance of *P. aeruginosa* to antibiotics (Mosharraf *et al.,* 2020). Additionally, *P. aeruginosa* expresses efflux systems, that produces antibiotic-inactivating enzymes, and modifies its target (Paladini and Pollini, 2019). Thus, resulting in the reduction of the concentration of the drugs.

2.5.2 Factors contributing to AMR.

Factors that contribute to the emergence of AMR includes the misuse and overuse of antimicrobials in developing nations (Zahid *et al.,* 2022, Akande-Sholabi and Ajamu, 2021, Collignon *et al.,* 2018). For example, poverty and limited access to health care can cause treatment to be delayed or not carried out properly, which can lead to the overuse or misuse of antibiotics (Dadi *et al.,* 2018). These actions put patients at risk of bacteria that are resistant to multiple antibiotics and limit the effectiveness of stronger antibiotics (Dolecek *et al.,* 2022). Furthermore, poor access to water and sanitation is also significant factor contributing to AMR. Studies have investigated the inhibitory effects of antibiotics on the growth and viability of pathogenic microorganisms, such as bacteria and fungi. However, the misuse and overuse of the antibiotics lead to reduced efficacy and consequently AMR. AMR have dramatically increased over the past century, posing a serious threat to the efficacy of many medical interventions, and having a significant impact on global public health, healthcare systems, and economies. As such green synthesized AgNPs with their unique properties, such as increased surface area and increased efficacy might be used effectively to combat AMR.

2.5.3 AgNPs as a possible solution to combat AMR

The precise mechanism by which Ag ions or AgNPs exert their antimicrobial effects remains incompletely understood. However, several studies have demonstrated that Ag ions have the ability to form complex structures through their binding with negatively charged proteins and nucleic acids. This interaction leads to alterations and deformations in the cell wall, membranes, and nucleic acids of the pathogen (Ndayishimiye *et al*., 2022). The research findings demonstrate that AgNPs can interfere with the integrity of the cell membrane, interfere with synthesis of nucleic acid, interrupt the structure of bacterial membrane, and leads to the release of reactive oxygen species (ROS). According to Hulikere and Joshi (2019), the antimicrobial properties of AgNPs are attributed to their ability to disrupt the cell membrane, thereby inducing the release of ROS that subsequently cause damage to DNA and proteins (Hulikere and Joshi, 2019).

AgNPs possess the ability to attach themselves to the bacterial cell wall, leading to their penetration into the cell (Khalandi *et al.,* 2017). This process induces the physical alterations in the cell membrane, and cause changes in its absorptivity and increase cellular permeability. The increased permeability of cells and release of their internal contents, caused by cellular disruption, is believed to be caused by the production of ROS by AgNPs (Takamiya *et al.,* 2016, Patra *et al.,* 2021). This can trigger oxidative stress and ultimately result in cell death (Florkiewicz *et al.,* 2019). The NPs can also interact with the nucleic

acids either DNA or RNA via diverse mechanisms, such as electrostatic interactions (Rayamajhi *et al.,* 2021, Cinteza *et al.,* 2018).

These interactions may interfere with the replication and transcription processes, resulting in disruptions in the synthesis of new nucleic acid strands (Satpathi *et al.,* 2021, Meenambal *et al.,* 2022). Additionally, these interactions may interfere with specific membrane proteins that play crucial roles in important biological processes such as cellular signalling, ion transportation, and receptor interaction resulting in cellular disruptions in microorganisms (Negahdari *et al.,* 2021). The combination of these mechanisms minimises the probability of microorganisms gaining resistance to AgNPs (Viet and Xo, 2016). AgNPs inhibit the development of resistance, which is common with conventional antimicrobial agents, by targeting multiple sites within microorganisms (Viet and Xo, 2016, Al-Ghamdi *et al.,* 2020, Tobin and Brenner, 2021).

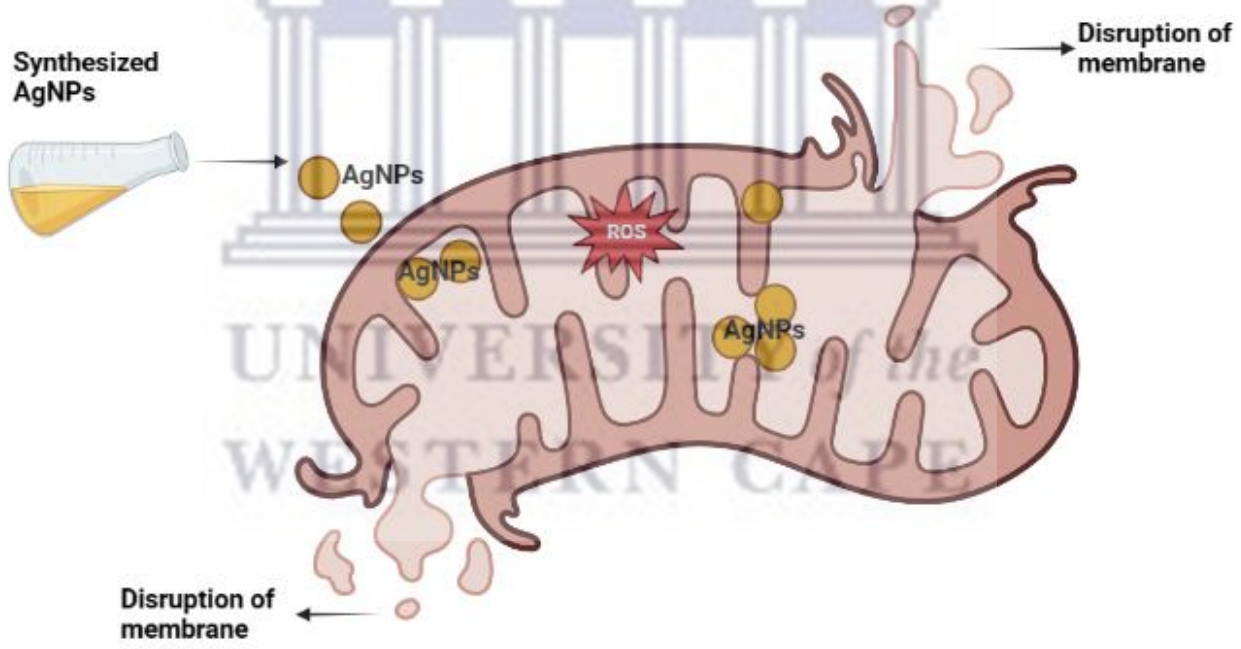


Figure 3.3: Mechanism of actions used by AgNPs to combat AMR.

CHAPTER 3

3. RESEARCH METHODOLOGY

Table 3. 1: Bacterial/fungal strains and their suppliers

Fungal/ bacterial strains	ATCC Numbers	Gram reaction	Supplier
Candida albicans	10231	Gram-positive fungus	ATCC
Klebsiella pneumonia	13883	Gram-negative bacteria	ATCC
Pseudomonas aeruginosa	27853	Gram-negative bacteria	ATCC
	35218	Gram-negative bacteria	ATCC
Staphylococcus aureus	25923	Gram-positive bacteria	ATCC
Methicillin-resistant staphylococcus aureus	33591	Gram-positive bacteria	ATCC

Table 3. 2: cell line used in this study

Cell line	Species	Source	Media
KMST-6	Human	Fibroblast	Complete DMEM

3.1 Preparation, synthesis and optimisation of CsTe-AgNPs

The CsTe-cocktail (freeze-dried) was received from Global Health Biotech (Pty) Ltd. The freeze-dried cocktail was weighed and mixed with deionised water (ddH_2O) and stored at -20 °C until required. For the synthesis of CsTe-AgNPs, 6.25 mg/mL extracts concentration was used for the optimisation. The CsTe-AgNPs were synthesized by heating 1 mM AgNO3 to the desired temperature for 5 minutes, followed by the addition of CsTe in 2 mL Eppendorf tubes using a 1:9 ratio. The changing colour from pale yellow to dark brown confirmed the formation of CsTe-AgNPs. Various parameters were optimised for the synthesis process. This included temperature (25 -90 °C), pH (3–11), silver nitrate ($AgNO_3$) concentrations (1–5 mM), plant cocktail concentrations (3.125 -100 mg/mL), reaction volume of CsTe-cocktail: $AgNO_3$ (1:1, 1:4, 1:7, and 1:9), and reaction time (0 – 24 hrs). All reactions were shaken at 750 rpm on an orbital shaker (Eppendorf Thermomixer Comfort, Hamburg, Germany). Thereafter, CsTe-AgNPs were washed thrice by centrifugation at 13,200 rpm for 30 minutes in order to remove excess unreacted plant extracts material. The supernatant was discarded, and the pellet was resuspended in ddH_2O and stored at 25 °C (room temperature in the dark).

3.2 Characterization of CsTe-AgNPs

In order to evaluate the physicochemical characteristics of the synthesized CsTe-AgNPs, various techniques were used. These techniques included Ultraviolet visible (UV-Vis) spectroscopy, Dynamic Light Scattering (DLS), High-Resolution Transmission Electron Microscope (HR-TEM), and Fourier-Transmittance Infrared (FT-IR) Spectroscopy to check the physicochemical properties of the synthesized AgNPs.

3.2.1 Ultraviolet Visible spectroscopy (UV-Vis)

UV-Visible spectroscopy technique is used for the structural characterization of NPs (Guzman *et al.,* 2012). The UV-Vis spectroscopy was utilised to measure the surface plasmon resonance (SPR) phenomenon, which is responsible for the optical properties of AgNPs. The CsTe-AgNPs sample was diluted to a final volume of 300 µL in a 96-well flat-bottom microtiter plate with sterile ddH_2O at a ratio of 1:10 (v/v). The formation of CsTe-AgNPs was confirmed using a POLARstar Omega microplate reader (BMG Labtech, Offenburg, Germany) at wavelengths ranging from 300 - 800 nm. The obtained data was then analysed with Omega Mars and Microsoft Excel.

3.2.2 Dynamic Light Scattering (DLS)

DLS technique are used to determine particle size and distribution of particles. (Ratan *et al.*, 2020). The hydrodynamic diameter, poly-dispersity index (PDI), and zeta potential (ζ-potential) of CsTe-AgNPs were measured utilising a Nano-ZS90 Zetasizer (Malvern Instruments Ltd., Malvern, UK). To measure hydrodynamic size, the synthesised and washed CsTe-AgNPs were placed in a 1:10 (v/v) ratio with sterile ddH_2O and transferred to a clean 10 mm optical density square cuvette. The hydrodynamic size was measured at a temperature of 25 °C and an angle of 90 °. In order to assess the ζ-potential of NPs, the previously diluted samples were transferred into Disposable Capillary Cell (DTS1070) cuvettes. The measurements were conducted at a voltage of 4 mV, with the temperature set at 25 °C and the angle at 90 °.

3.2.3 High Resolution-Transmission Electron Microscopy (HR-TEM)

The morphology and core size of biogenic CsTe-AgNPs were determined using an FEI Tecnai G2 20 field-emission gun (FEG) HRTEM (Hillsboro, OR, USA) operating in bright field mode at 200 kV. The samples were prepared by loading aqueous CsTe-AgNPs onto a carbon-coated copper grid, allowing them to dry for 10 minutes under a Xenon lamp, and then analysed under a microscope. Using an EDX

liquid nitrogen-cooled lithium-doped Silicon detector, the elemental composition of AgNPs was characterised. In addition, HR-TEM was also used to perform Selected Area Electron Diffraction (SAED) analyses.

3.2.4 Fourier Transmittance-Infrared (FT-IR) spectroscopy

The functional groups in CsTe and CsTe-AgNPs were determined by FT-IR analysis using a PerkinElmer Spectrum One FTIR spectrophotometer (Waltham, MA, USA) at a wavelength range of 4000 – 500 cm^{-1}. For the sample preparation, dried CsTe-extracts and CsTe-AgNPs were combined with Potassium bromide (KBr) using a pestle and mortal. The powdered mixture was pelletized after being compressed into a circular disc. KBr discs were used as background correction.

3.3 Stability test for CsTe-AgNPs

The stability assessment of CsTe-AgNPs was conducted by subjecting them to various biological media, including Müller-Hilton Broth (MHB), Potato Dexton Broth (PDB), Dulbecco's Phosphate-Buffered Saline (DPBS) and Dulbecco Modified Eagle's Medium (DMEM) with or without complete supplementation with Fetal bovine serum (10% FBS). A sterile Eppendorf tube was used to hold a 500 mL aqueous solution of CsTe-AgNPs mixed with 500 µL of the respective medium in a 1:1 ratio. The tube was then incubated at a temperature of 37 °C for a duration of 24 - 72 hrs. The stability of CsTe-AgNPs was subsequently assessed through the measurement of UV-Vis spectra using a POLARstar Omega microplate reader at wavelengths ranging from 300 - 800 nm.

3.4 Antimicrobial activity

3.4.1 Culturing protocol

Agar plates and media were prepared by weighing appropriate nutrient media following manufacturer instructions. The agar/media was mixed with distilled water, then sterilised by autoclaved at 121 °C for 20 minutes. After autoclaving the agar was allowed to cool down to 40 - 45 °C. Sterilised agar was poured into sterile plates in the laminar flow and allowed to solidify. The prepared media and plates were stored at 4 °C. Using a sterile loop, microbial culture was gently spread on the surface of the agar plate. The inoculated agar plates were incubated at 37 °C for 24 -72 hrs. The presence of colonies was monitored.

3.4.2 Agar well diffusion

The bacterial/ fungal cultures were standardised to a 0.5 McFarland standard by adjusting the optical density at 600 nm, using a POLARstar Omega microplate reader (BMG Labtech, Offenburg, Germany) to a range of 0.08 - 0.1. The microorganisms investigated include *Staphylococcus aureus*, *Methanol-resistant Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Candida albicans.* At their 0.5 McFarland standard, the microorganisms were swabbed across the entire surface of Mueller Hinton Agar (MHA) plates or Potato Dexton Agar (PDA) plates for *C. albicans*. Then, a well of 6 to 8 mm in diameter was aseptically punched into the agar using a sterile yellow tip, and the treatments (50 µL) were introduced into the wells. Negative control Mueller Hinton Broth (MHB) or Potato Dexton Broth (PDB) was added, followed by positive control ciprofloxacin/ cycloheximide (15 or 25 µg/mL), plant extracts (12.5 mg/mL), and AgNPs (540 µg/ mL) in triplicates in their respective wells. The inhibition zones were measured in millimetres (mm) after 24 hrs of incubation at 37 °C.

3.4.3 Microbroth dilution assay

Microdilution assay was used to determine the Minimum Inhibition Concentration (MIC) of CsTe-AgNPs and aqueous extracts. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* were used to determine MIC. The MHB / PDB were inoculated with a single bacterial or fungal colony, were cultured by shaking at 400 rpm for 1 – 2 hrs at 37 °C. Thereafter, using UV-Vis microplate reader, bacterial/ fungal suspensions were then standardised to 0.5 McFarland (0.08 - 0.1) at OD_{600} nm and diluted in a 1:150 ratio. About 50 µL of CsTe-AgNPs and extracts were added to a 96-well plate at decreasing concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg/mL). Approximately 50 µL of the microbial suspension was added to each well. The 96-well plate was properly sealed and incubated at 37 °C for 24 hrs. After incubation, the MIC was determined using a spectrophotometer at a 600 nm wavelength. Each well received 10 µL of Alamar Blue, and the plate was incubated in the dark for three hours. Monitoring the colour change for the presence or absence of viable bacteria. The experiment was repeated three times, and the average absorbance ratios were calculated and used to generate bar graphs.

3.5 Antioxidant of AgNPs

The free radical scavenging activity was measured using 1, 1 diphenyl-2-picryl-hydraxyl (DPPH) assay conducted according to the previously reported protocol with slight modifications (Khorrami *et al.,* 2018). A volume of 100 µL of DPPH solution, with a concentration of 0.3 mM in methanol, was carefully

dispensed into individual wells of a 96-well plate. Subsequently, varying concentrations of CsTe-AgNPs, CsTe-extracts, and ascorbic acid ranging from 3.125 - 400 µg/mL were introduced into individual wells. Methanol was used as a negative control. Therefore, the plate was incubated in a dark environment for a duration of 30 min at a room temperature of 25 °C in the dark. The absorbance of the sample was subsequently evaluated at a wavelength of 517 nm using a UV–Vis spectrophotometer. The calculation of the inhibition ratio was performed using the following formula: the percentage of DPPH radical-scavenging activity (% DPPH radical-scavenging) was determined by subtracting the absorbance of the sample in DPPH solution (A_{test}) from the absorbance of the control solution ($A_{control}$), dividing the result by $A_{control,}$ and then multiplying by 100.

$$\% \text{ DPPH radical scavenging} = \frac{A\ control - A\ test}{A\ control} \times 100$$

3.6 Cytotoxicity of green synthesized CsTe-AgNPs

The effects of the green synthesized CsTe-AgNPs were investigated on normal skin cells (KMST-6) using MTT assay as according to a previously reported protocol with slight modification (Fadaka *et al.*, 2022). The cells were seeded in 96 well plates at a cell density of 1×10^5 cells/mL, with 100 µL of the cell suspension per well. The plates were incubated at 37 °C for 24 hrs and treated with increasing concentration of CsTe-AgNPs, CsTe-extract and allantoin which was used as a standard (0 - 50 µg/mL) and prepared in their respective media. 10% dimethyl sulfoxide (DMSO) solution was used as a positive control, while a negative control was established using untreated cells. The experimental procedures were conducted in triplicates and the samples were incubated at a temperature of 37 °C for a duration of 24 hrs.

Thereafter, the media was removed from the 96 well plate and replaced with fresh media comprising 10% of MTT dye at a concentration of 0.5mg/mL with a total volume of 100µL being added to each individual well. Subsequently, the plate was sealed with foil and subjected to incubation at a temperature of 37 °C for a duration of 3 hrs. Following the incubation period, the MTT solution was removed from all wells. A 100µL volume of DMSO was introduced into each well and subjected to incubation at a temperature of 37 °C for a duration of 30 min. Thereafter, the measurement of absorbance was performed at a wavelength of 570 nm.

CHAPTER 4

4. RESULTS AND DISCUSSION

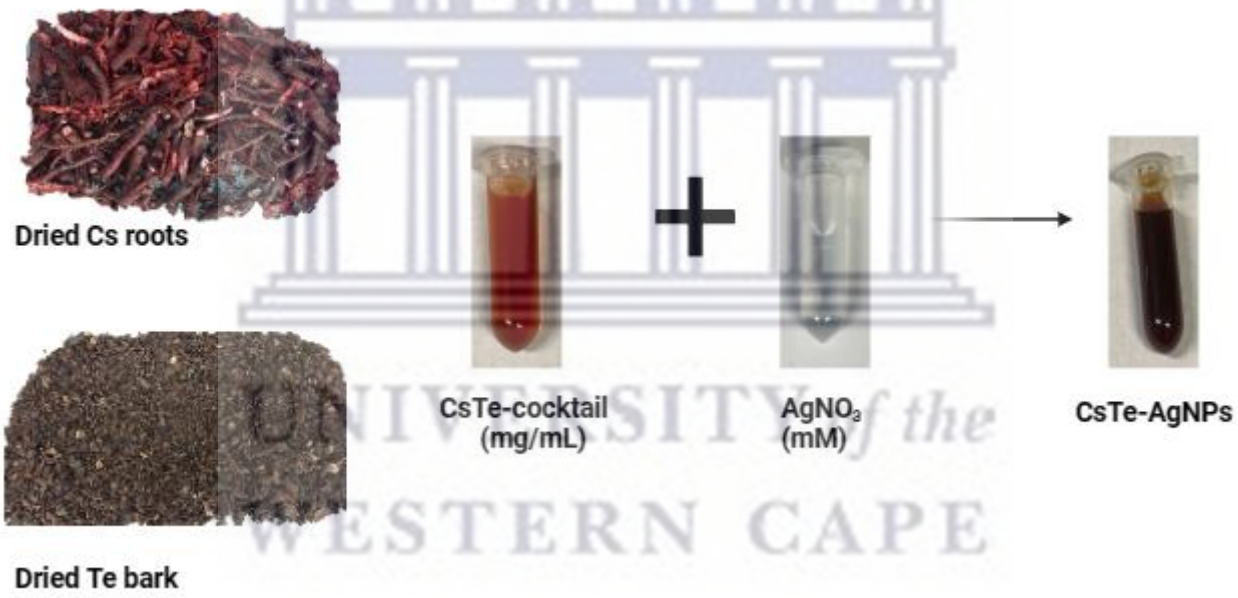
In recent times, there has been an increasing focus on the green synthesis of metallic NPs due to its potential for sustainable and eco-friendly production of NPs. The use of plant extracts for the green synthesis of AgNPs is recognised as a significant approach, serving as an alternative for conventional chemical and physical techniques (Velidandi *et al.,* 2020). This approach presents multiple benefits, including its simplicity, cost-effectiveness, and utilisation of non-toxic reducing agents derived from plant extracts. The green synthesis of AgNPs using plant extracts has a variety of benefits, including being affordable, easily scalable for large-scale production, and lacking the need for stabilisers like polyethylene glycols (Velidandi *et al.,* 2020). Additionally, a variety of phytochemicals found in plant extracts can efficiently reduce silver ions to AgNPs, making them the perfect reducing agents for synthesis (Ramalingam and Udayaprakash, 2019, Rajeshkumar and Bharath, 2017). Several studies have examined the environmentally friendly synthesis of AgNPs using various plant extracts, such as fruit, stem, roots, bark and leaf extracts (Devatha and Thalla, 2018). In this study, AgNPs were successfully green synthesized using root extract of *C. sepiaria* and bark extract of *T. elegans* cocktail as the reducing and stabilising agents.

The use of plant extracts for the green synthesis of AgNPs has been demonstrated to possess antioxidant and antimicrobial properties, thereby increasing their potential applications in biomedical fields (Mata *et al.,* 2015). This approach offers several notable benefits, such as economic viability, scalability, antimicrobial agent, and antioxidant efficacy. Furthermore, the characterization of AgNPs synthesized through environmentally friendly techniques performed in order to ensure their high standard, durability, and efficacy. Various characterization techniques, including UV-Visible spectroscopy, DLS, HR-TEM, and FT-IR, were used and they offer significant insights into the size, shape, structure, stability, and chemical composition of AgNPs.

4.1 Optimisation and biosynthesis of CsTe-AgNPs

As part of the normal process for synthesizing AgNPs, various reaction parameters, including temperature, pH, the concentrations of $AgNO_3$ and the plant extracts, as well as reaction time were optimised. Each plant extract differs from another in terms of its ability to synthesize NPs. In this study, synthesis of AgNPs was achieved using the freeze-dried C. *sepiaria* and *T. elegans* aqueous extracts. The

formation of CsTe-AgNPs was preliminarily confirmed by the rapid colour change of $AgNO_3$ from colourless to yellow to brown colour after the addition of CsTe-cocktail extracts to 1 mM $AgNO_3$ solution (**Scheme 1**). The colour change is attributed to the SPR, which is due to the collective oscillation of electrons of metallic NPs in resonance with the lightwave (Ajitha *et al.,* 2015). Typically, the SPR for AgNPs occurs in the 400 – 450 nm range, and its width and position is strongly determined by several factors such as size, shape, electron density, and other properties (Gontijo *et al.,* 2020, Xia and Halas, 2005). These factors can be fine-tuned and improved by altering reaction parameters including temperature, pH, extracts and silver salt concentrations during synthesis (Wisam and Haneen, 2018, Akintelu *et al.,* 2020). Therefore, in order to obtain AgNPs with a tuneable shape and size, the CsTe-AgNPs synthesis was optimized by adjusting various reaction parameters. The synthesized CsTe-AgNPs were then characterized using UV-Vis technique.



Scheme 1: Synthesis of CsTe-AgNPs**.**

4.2 Establishment of optimum conditions for the synthesis of CsTe-AgNPs

4.2.1 Effects of pH on the synthesis of CsTe-AgNPs

The pH of the solution is an important factor that influences the size, shape, and stability of the NPs. As shown in **Figure 4.1**, the CsTe-AgNPs synthesis was performed at various pH's ranging from acidic to basic pH (3 to 11). The reduction of Ag^{+} to Ag^{0} by CsTe was evidenced by the UV-Vis spectra showing well-defined SPR peaks ranging between 403 – 457 nm. No AgNPs were synthesized at pH 3 at all the temperatures, suggesting that extremely low pH hinders the formation of AgNPs. At pH 4, synthesis of NPs was only successful at higher temperatures (70 - 90 °C), no SPR peak or any colour change was observed at lower temperatures (25 – 50 °C). Increasing the pH of the CsTe cocktail resulted in an increase in the absorbance values as observed from pH 5 to pH 11, at all temperatures used. Although, synthesis of AgNPs at pH 5 was successful at all temperatures (25 – 90 °C), lower temperatures (25 – 50 °C) were associated with broader peaks, possibly suggesting variation in particle size or shape within a sample. Interestingly, at pH 6 (normal pH of the extracts) synthesis of AgNPs was successful at all temperatures (25 – 90 °C) as indicated by the SPR peak; amongst all the temperatures, 70 °C allowed for the synthesis of narrow and sharper peak. Moreover, the remaining pH (7 – 11) allowed for the synthesis of AgNPs at all temperatures (25 – 90 °C).

The findings indicates that as the pH of the solution increases, the intensity of the SPR peak of AgNPs also increases, resulting in sharper and narrower peaks for the NPs. This change in SPR band value might be encouraged by the alkaline environment that the NaOH creates in the solution which facilitates faster Ag + reduction and the production of AgNPs (Chiguvare *et al*., 2016). The highest intensity of the SPR peak was observed between 410 – 415 nm for pH 11, indicating a higher synthesis yield. Moreover, the CsTe-AgNPs at pH 11 exhibited a sharp, narrow and intense SPR peak, suggesting the synthesis of uniform and small AgNPs. At higher pH ranges the large number of functional groups available for silver binding facilitated a higher number of AgNPs to bind and subsequently form NPs with a smaller diameter due to increased nucleation (Veerasamy *et al.,* 2011, Madiehe *et al.,* 2022).

The results are corroborated by previous studies which have shown that acidic conditions suppress the formation of AgNPs, whereas the basic conditions enhance their formation (Velgosová *et al.,* 2016,

Veerasamy *et al.,* 2011, Singh and Srivastava, 2015). Therefore, pH 11 was chosen as the optimum pH for the synthesis of CsTe-AgNPs.

4.2.2 Effects of temperature on the synthesis of CsTe-AgNPs

Temperature is another important factor that has a significant effect on the synthesis of NPs. Hence, to investigate the influence of temperature on AgNPs synthesis, the reaction was carried out at various temperatures, ranging from of 25 to 90 °C. As shown in **Figure 4.1**, all temperatures successfully synthesized CsTe-AgNPs. AgNPs formed at lower temperatures (25, 37 and 50 °C) exhibited broad SPR peaks, possibly suggesting the formation of large and polydispersed NPs. Furthermore, the AgNPs formed at these temperatures had the lowest intensities, indicating lower AgNPs yields. In contrast, higher temperatures (70 – 90 °C) favoured the formation of CsTe-AgNPs with higher yields associated with the sharp, narrow and intense SPR peaks at 403 – 415 nm.

Increasing the temperature, up to 70 °C, increased the bio-reduction as well as the rate of AgNPs synthesis, indicating that the rate of formation of AgNPs was related to the incubation temperature of the reaction. As a result, 70 °C was then selected as the optimum temperature for the synthesis of CsTe-AgNPs. While CsTe-AgNPs synthesized using pH 11 at 70 °C exhibited a well-defined SPR peak at 409 nm and had the highest intensity; CsTe-AgNPs synthesized at pH 6 had a blue shifted SPR at 404 nm, suggesting the formation AgNPs that are highly monodispersed and smaller in size. Therefore, the optimization of subsequent parameters were carried forward with pH 6 and 11. The utilization of pH 6, which is the natural (unpH'd) CsTe cocktail pH, is in accordance with previous studies that have reported synthesis of spherical, small and monodispersed AgNPs using plant extracts at their natural pH's (Mehata, 2021).

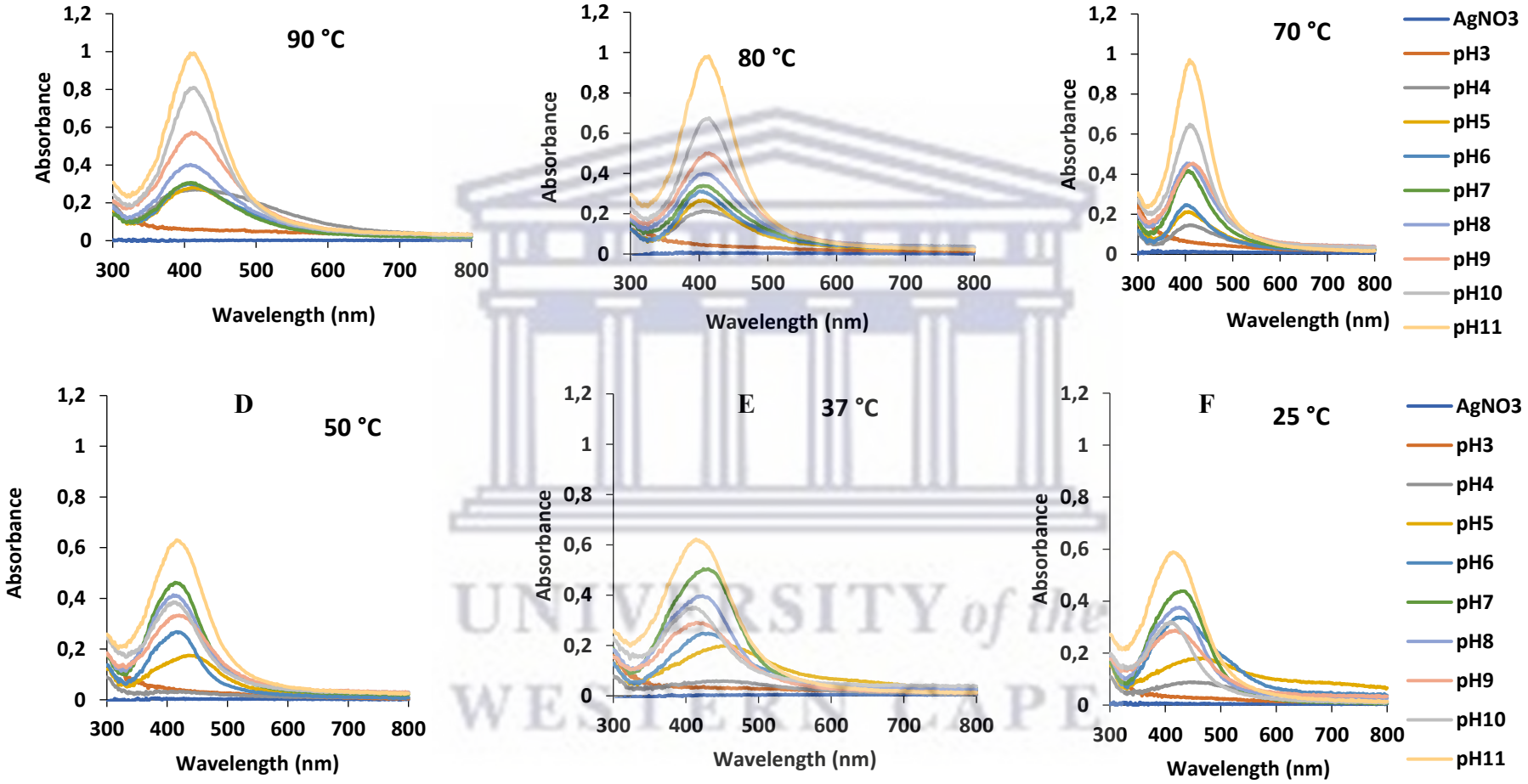


Figure 4.1: UV-Vis spectra of CsTe-AgNPs synthesised at various temperatures (A - F, representing 90, 80, 70, 50, 37, 25 °C, respectfully) and pH's (3, 4, 5, 6, 7, 8, 9, 10, and 11)

4.2.3 Effects of plant extracts concentration in the synthesis

In the synthesis of NPs, the types of plants and the primary bioactive compounds are associated with the rate at which silver ions are reduced. Various CsTe-extracts concentrations (3.125, 6.25, 12.5, 25, 50, and 100 mg/mL) and constant Ag^0 of 1 mM were used to determine the maximum synthesis of CsTe-AgNPs at pH 6 and pH 11 (**Figure 4.2**). A low SPR peak at 412 and 408 nm was observed for CsTe-AgNPs at pH 6 and pH 11 at 3.125 mg/mL, indicating a low synthesis yield and this could be due to insufficient capping and reducing agents (Nagar *et al.,* 2016, Meva *et al.,* 2016). According to the nucleation and growth model, low concentration of active phytochemicals is associated with reduced nucleation and increased formation of a small number of nuclei; thus, favouring the growth of larger NPs (Skandalis *et al.,* 2017).

Furthermore, an increase in plant concentrations above 3.125 mg/mL resulted in increased in the intensities of the SPR peak. CsTe-AgNPs exhibited narrow, sharp, and intense SPR peaks between 400 and 406 nm for pH 6 (**Figure 4.2A**) and pH 11 (**Figure 4.2B**) at 12.5 mg/mL, which suggested the formation of smaller, uniform, and spherical NPs. This can be attributed to the AgNPs' surfaces being completely covered by the phytochemicals from the CsTe extracts cocktail. Although, AgNPs synthesized using 25 mg/mL CsTe at pH 11 were both narrow and sharp at an SPR peak at 399 nm, there was an observed decline in the intensity of AgNPs with the increase of the extracts concentration from 12.5 to 25 mg/mL, which might indicate some agglomeration of the AgNPs.

Moreover, as the concentration increased from 25 - 100 mg/mL, the SPR peak shifted towards longer wavelengths (from 399 to 417 nm), and the absorbance values also decreased, suggesting the formation of larger or aggregated AgNPs. These results are consistent with several studies that have hypothesized that often higher concentration of plant extracts lead to agglomeration and formation of large particles, with excess reducing agents potentially causing secondary reduction process on the surface of the preformed nuclei (Azizi *et al.,* 2017). Therefore, 12.5 mg/mL was chosen as the optimum concentration for synthesis of CsTe-AgNPs at pH 6 and pH 11 (**Table 4.1**).

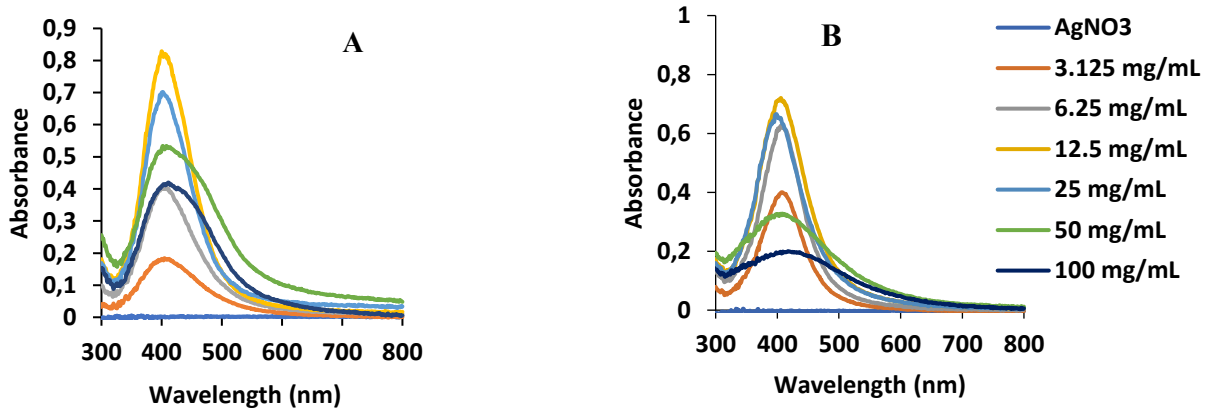


Figure 4.2: Changes in the UV-Vis absorption spectra of CsTe-AgNPs, synthesized using varying plant extracts concentrations (100, 50, 25, 12.5, 6.25 and 3.125 mg/mL) and constant AgNO3 concentration of 1 mM. A-CsTe-AgNPs at pH 6 and B-CsTe-AgNPs at pH 11.

Table 4. 1: Representing highest SPR peaks and absorbance of CsTe-AgNPs (pH 6 and pH 11) at different reactions. Various concentrations of CsTe cocktail (3.125 to 100 mg/ mL) were incubated with 1mM AgNO3.

	AgNPs pH 6		AgNPs pH 11	
CsTe-cocktail concentration (mg/mL)	**Wavelength (nm)**	**Absorbance**	**Wavelength (nm)**	**Absorbance**
3.125	412	0.182	408	0.400
6.25	407	0.406	406	0.626
12.5	400	0.829	406	0.720
25	402	0.702	399	0.665
50	414	0.530	408	0.327
100	411	0.420	417	0.200

4.2.4 Effects of $AgNO_3$ on the synthesis of CsTe-AgNPs

In the synthesis of metallic NPs, the amount of the metal precursor present is very important. Numerous studies have demonstrated that 1 mM and 3 mM $AgNO_3$ concentrations are suitable for the synthesis of biogenic AgNPs (Logeswari *et al.,* 2015, Ahsan *et al.,* 2020). Therefore, the concentration of $AgNO_3$ (1 - 5 mM) were evaluated in the synthesis of CsTe-AgNPs. As shown in **Figure 4.3**, CsTe-AgNPs synthesized at 1 mM $AgNO_3$ for pH 6 CsTe cocktail exhibited a narrow and sharp SPR peak at 412 nm, indicating synthesis of smaller and uniform AgNPs. In contrast, an increase in $AgNO_3$ concentrations (2 – 5 mM) resulted in the synthesis of CsTe-AgNPs that exhibited broad SPR peaks ranging between 414 – 420 nm, suggesting the formation of AgNPs that are polydisperse and larger in size.

For pH 11, CsTe-AgNPs synthesized at 1 mM $AgNO_3$ displayed a weak intense SPR peak at 412 nm, whereas sharp, narrow and intense SPR peaks at 412 nm was observed at 2 and 3 mM $AgNO_3$. Interestingly, when the silver concentration was increased to 4 mM, the SPR peak shifted from 412 to 410 nm, indicating a blue shift. Despite having a blue shift and narrower SPR peak at 4 mM, AgNPs displayed longer wavelength tail which might indicate anisotropic growth. AgNPs synthesized at 5 mM $AgNO_3$ displayed a broad, and weak intense SPR peak at 412 nm, and had low absorbance. This is because at high $AgNO_3$ concentrations, the key biomolecules responsible for reduction are saturated with silver ions, and no site is available for further growth of NPs (Singh *et al.,* 2013). This study's findings are corroborated with study by Kumar and Yadav, where in nucleation was complete at 3 mM and 4 mM, AgNO3 concentration, further increment beyond 3 and 4 mM AgNO3 resulted in the decrease in NPs synthesis (Alim-Al-Razy *et al.,* 2020). Based on the results, 1 mM and 3 mM were selected as optimum $AgNO_3$ concentrations for pH 6 and 11, respectively.

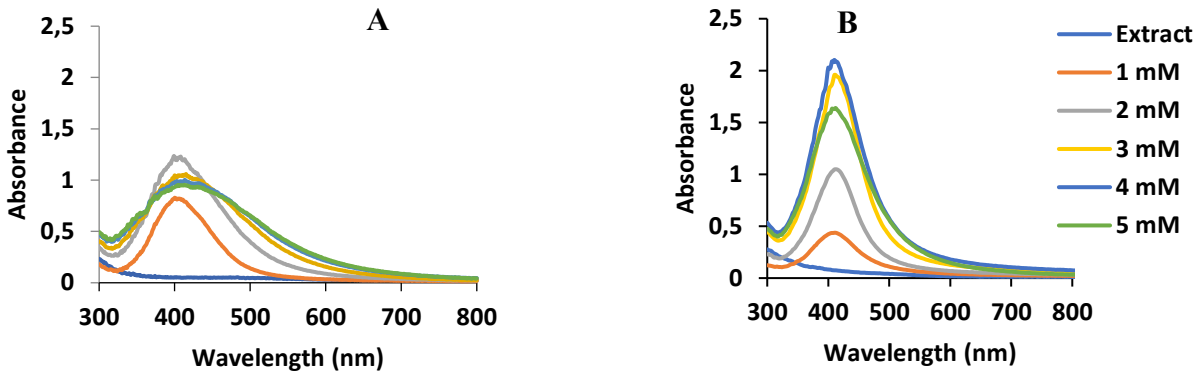


Figure 4.3: Changes in the UV-Vis absorption spectra of CsTe-AgNPs synthesized at various AgNO3 concentrations (1, 2, 3, 4 and 5 mM). A-CsTe-AgNPs at pH 6, B-CsTe-AgNPs at pH 11.

4.2.5 Effects of ratio optimisation

The effects of the volume ratios of $AgNO_3$ to CsTe extract on the synthesis of CsTe-AgNPs were also optimized. As shown in **Figure 4.4**, using equal volumes of $AgNO_3$ to CsTe (1:1) resulted in NPs with low synthesis yield at pH 6 (**Figure 4.4A**), whereas no NPs were formed at pH 11 (**Figure 4.4B**). This may be due to the slow reduction of Ag^+ to Ag^0. However, adding higher volumes of $AgNO_3$ to CsTe extract (1:9), resulted in a more intense absorption peak. Among the various concentrations used in this study, the ratio 1:9 (CsTe: to $AgNO_3$) produced SPR peaks that were well-defined and narrower with the highest SPR peak of 1.5. The results are consistent with those of a previous study by Dada *et al.,* 2018, in which the extract stabilized and converted silver salt to AgNPs at a 1:9 ratio.

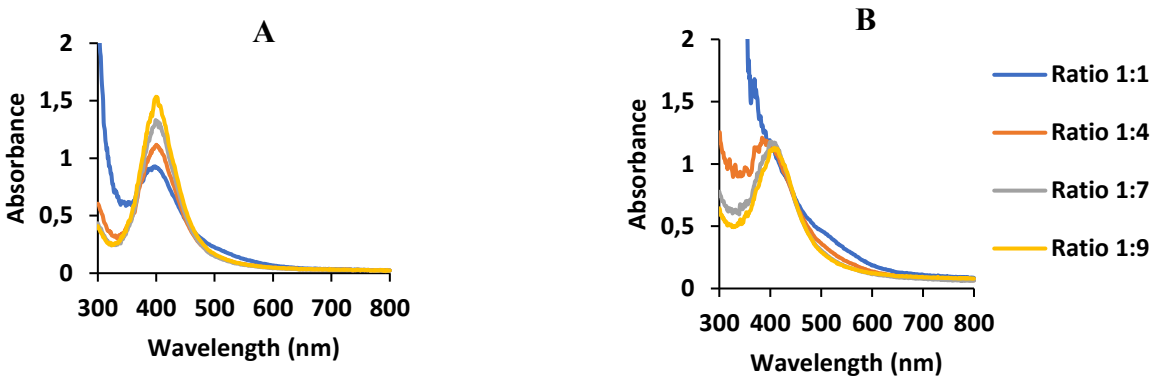


Figure 4.4: Changes in the UV-Vis absorption spectra of CsTe-AgNPs, synthesized using varying CsTe extracts and $AgNO_3$ volume ratios. A-CsTe-AgNPs at pH 6. B-CsTe-AgNPs at pH 11.

4.2.6 Reaction time optimisation

The effect of synthesis time was examined by monitoring the reaction at various time points. As shown in **Figure 4.5A**, the results demonstrate that the absorbance of CsTe-AgNPs increases with time to a certain point and starts to decline. **Figure 4.5A** shows CsTe-AgNPs synthesized at pH 6 to have a slight blue shift occurring at an SPR peak, transitioning from 411nm at 0.5 minutes to 403 nm at 120 minutes. Possibly indicating the decrease in particle size (Darroudi *et al*., 2011). In addition, the results reveal a constant reaction from 120 to 840 minutes, indicating that no silver remained for further reaction. However, further increments beyond 840 minutes resulted in a decrease in absorbance at an SPR peak of 406, possibly suggesting agglomeration of AgNPs. As a result, two hours was chosen as the optimal synthesis time. As seen in **Figure 4.5B,** CsTe-AgNPs synthesized at pH 11 exhibited a declining SPR peak from 417 at 10 minutes to 413 nm at 120 minutes. Increasing the time to 1440 minutes resulted in agglomeration of the AgNPs. For that reason, two hours was chosen as the optimal amount of time to ensure the reduction process is complete for both CsTe-AgNPs at pH 6 and pH 11.

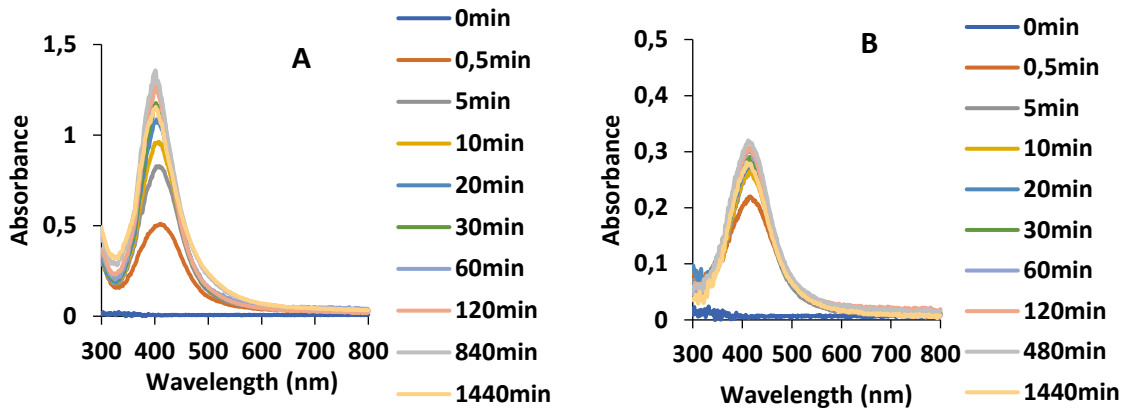


Figure 4.5: Changes in the UV-Vis absorption spectra of CsTe-AgNPs, synthesized at various time points (0 to 1440 min). A-Represent synthesis of AgNPs at pH 6. B-Represent synthesis of AgNPs at pH 11.

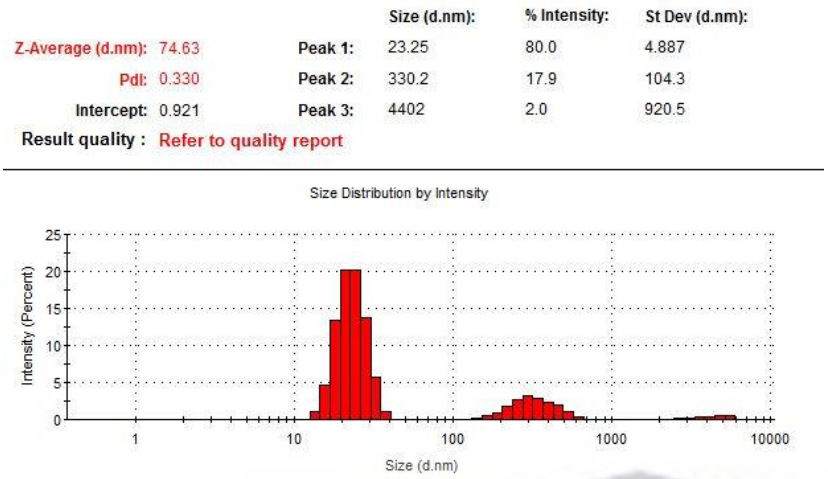
4.3 Characterisation of CsTe-AgNPs

4.3.1 DLS analysis

The DLS analysis was used to characterise the size distribution, PDI and ζ-potential of the CsTe-AgNPs in solution. As seen in **Figures 4.6,** the average hydrodynamic size of the optimised CsTe-AgNPs synthesized at both pH 6 and pH 11, respectively (23 ± 12.26 and 138 ± 2.086 nm). The CsTe-AgNPs displayed differences in the hydrodynamic sizes at pH 6 and pH 11, this dispersity might possibly be attributed due to various factors including the water molecules and other constituents in the solution including pH of a solution which might have several effects in the measurements. The size distribution by intensity of CsTe-AgNPs at pH 6 and pH 11, was displayed at 80 and 98.7 % respectively.

The PDI of a sample measures the uniformity of NPs. It is a crucial factor to take into account and may have an impact on the chemistry of surface conjugation and NP aggregation. The calculated PDI value of CsTe-AgNPs at pH 6 and pH 11 was 0.271 ± 0.049 and 0.322 ± 0.043, respectively. According to the International Organization for Standardization (ISO) states that PDI values > 0.7 denote a broad size distribution, whilst PDI values of < 0.5 denote a more monodisperse distribution (Majoumouo *et al.,* 2019). Thus, the outcome of this study shows that the synthesized CsTe-AgNPs were monodispersed.

A.



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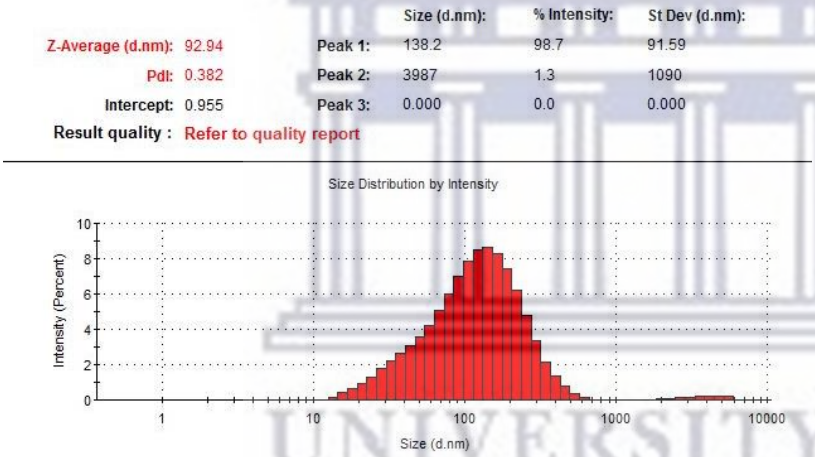


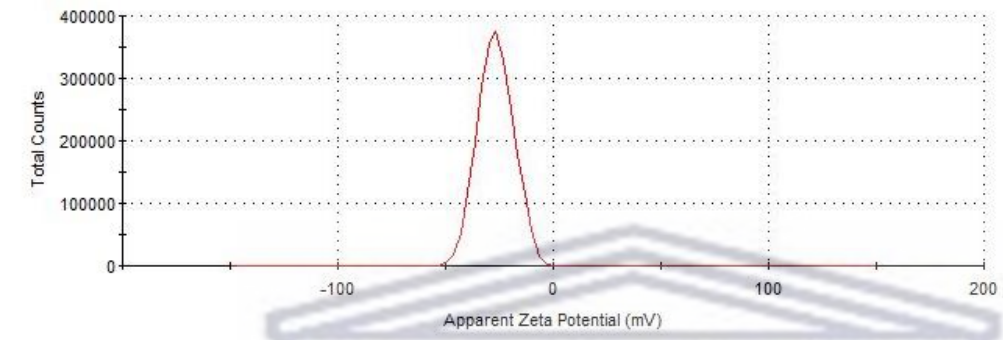
Figure 4.6: The hydrodynamic size and PDI analysis of the CsTe-AgNPs. A- CsTe-AgNPs at pH 6, B- CsTe-AgNPs at pH 11.

4.3.2 The ζ-potential of CsTe-AgNPs

The ζ-potential, quantifies the electrostatic potential of the particle, and determine their stability within a dispersion medium (Esmaeilzadeh *et al.,* 2022). **Figures 4.7** show the average ζ-potential of the optimised CsTe-AgNPs synthesized at both pH 6 and pH 11, respectively (-20 ± 0.583 and -24.9 ± 0.705 mV). Thus, indicating that the CsTe-AgNPs are highly stable at both pH 6 and pH 11. According to various studies, NPs with ζ-potential withing ± 30 mV, shows that there is an electrostatic repulsive force

to prevent aggregation of NPs (Hwang *et al.,* 2018, Tang *et al.,* 2017, Rasmussen *et al.,* 2020). Moreover, this range suggests that the surface charge of the NPs is stable, which decreases the likelihood of interactions that may result in aggregation (Mohamad Hanafiah *et al.,* 2023).

A.



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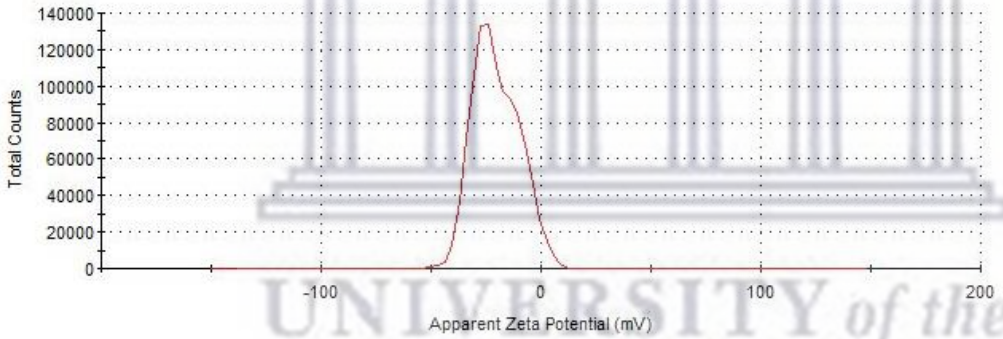


Figure 4.7: The ζ-potential of the CsTe-AgNPs. A- CsTe-AgNPs at pH 6, B- CsTe-AgNPs at pH 11.

4.3.3 HR-TEM analysis of CsTe-AgNPs

As seen in **Figure 4.8**, the CsTe-AgNPs had an average core size of approximately 14 ± 2.953 nm at pH 6 and 7 ± 3.849 nm at pH 11. HR-TEM analysis revealed the CsTe-AgNPs were spherical and monodispersed, without any sign of agglomeration. The HR- TEM findings display the mean particle size was much smaller than the DLS measurement at pH 6 and pH 11. The variation between the analysis may be because DLS analysis measures both the particles size and the surrounding solvent molecules

thus yielding NPs with bigger size distribution (Xu *et al.,* 2007, Florkiewicz *et al.,* 2021), whereas HR-TEM only measures the core size of the NPs. Moreover, the size discrepancy between the core and hydrodynamic diameters may also be attributed to the presence of phytochemicals on the surface of the AgNPs, which may significantly yield bigger size than the actual metal core size (Simon *et al.,* 2021).

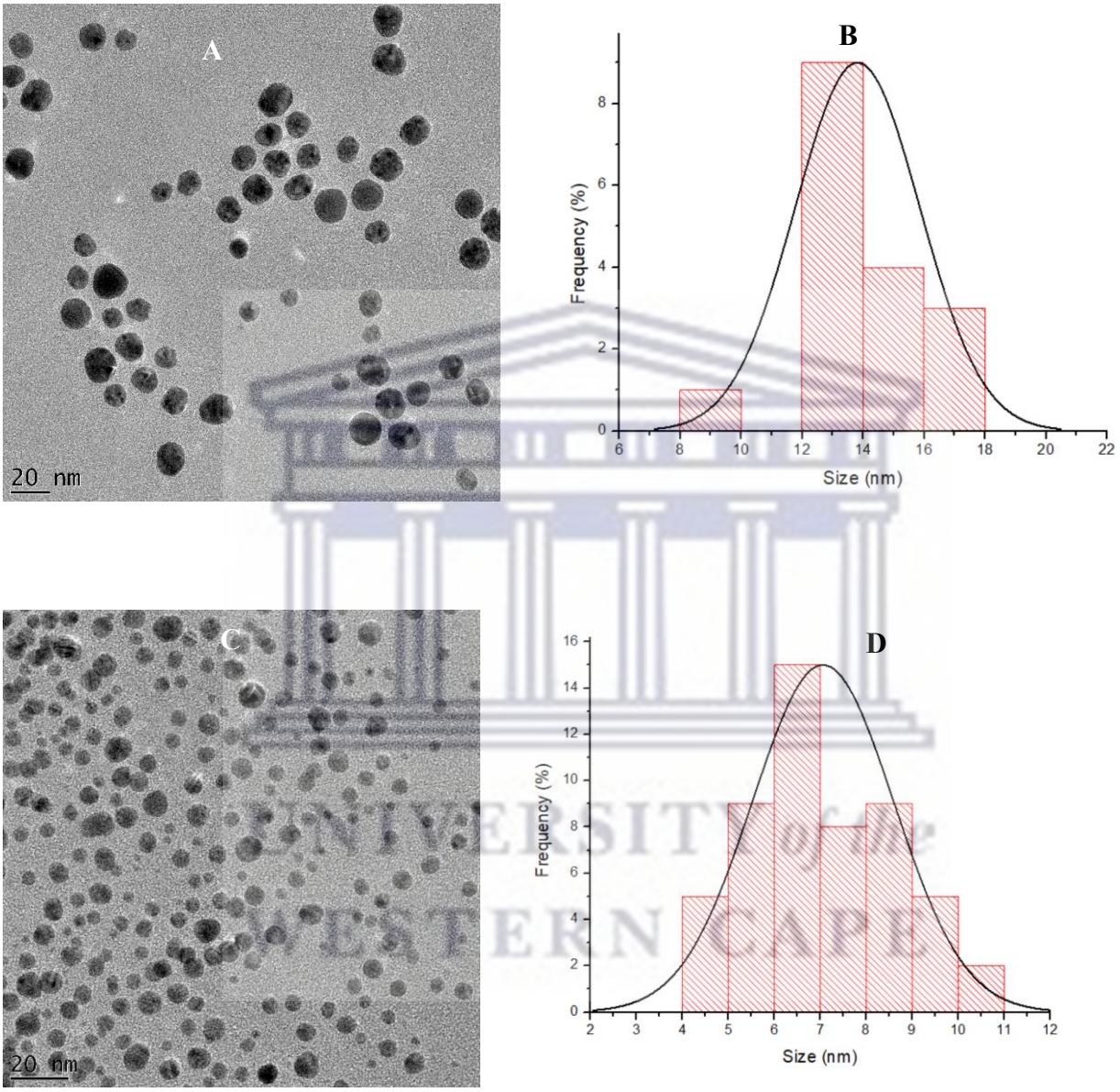
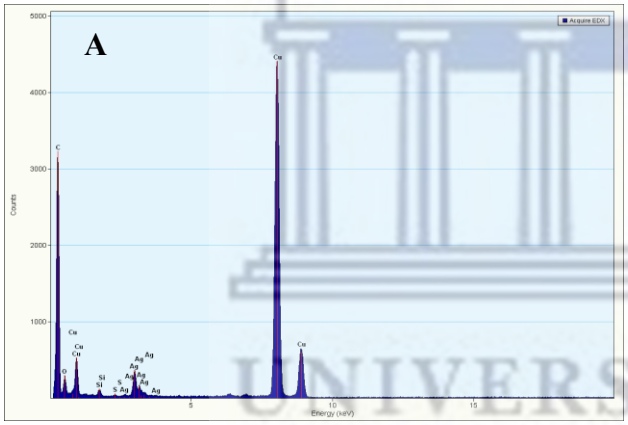
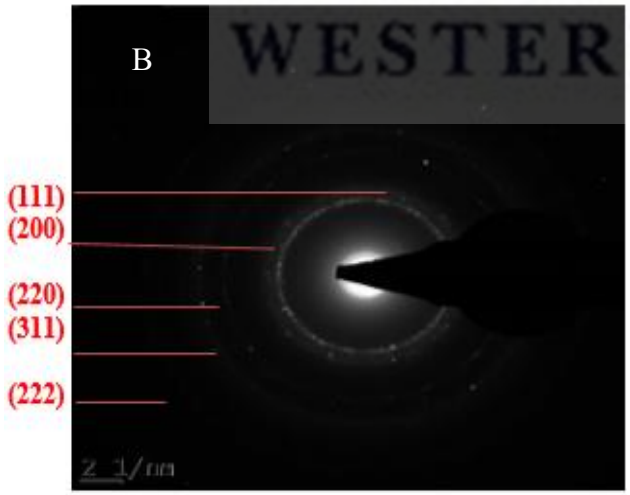


Figure 4.8: The HR-TEM data of CsTe-AgNPs. A & C- Core size of CsTe-AgNPs at pH 6 and pH 11. B & D- histogram chart of the CsTe-AgNPs at pH 6 and pH 11.

4.3.4 EDX and SAED analysis

CsTe-AgNPs contain silver, according to the elemental composition of NPs as revealed by EDX analysis (**Figures 4.9**). The optical adsorption peak at 3 keV, which is typical of the absorption of metallic silver nano crystallites due to surface plasmon resonance, is caused by the presence of silver (Shahverdi *et al.,* 2007). The presence of silicon and copper are due to the grid used, whereas the presence of carbon and oxygen is a result of the reduction and stabilization of the AgNPs by phytochemicals present in the extract (Tyavambiza *et al.,* 2021). These results indicated that CsTe-AgNPs synthesized at pH 6 and pH 11 contained silver ions. **Figures 4.9 B** and **D** show the selected area electron diffraction (SAED) patterns of CsTe-AgNPs. The observed rings were present at various phase crystals at (111), (200), (220), (311), and (222).





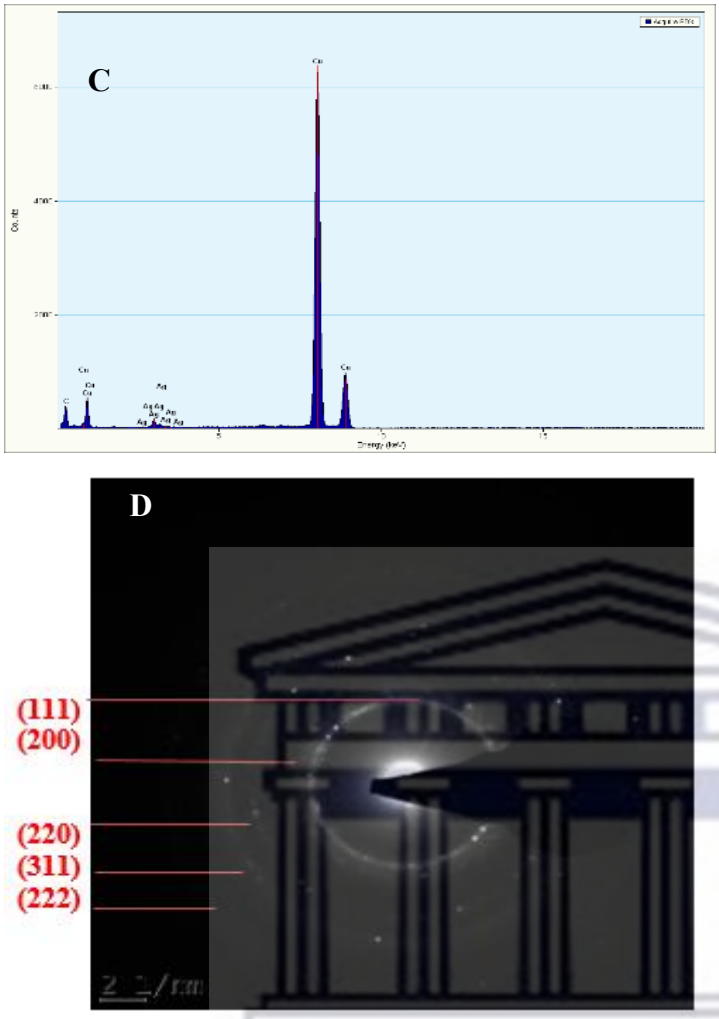


Figure 4.9: A & C-EDX data showing elemental composition of AgNPs. B & D-SAED pattern of AgNPs at pH 6 and 11, respectively.

4.3.5 FT-IR analysis

FTIR analysis was performed to identify the functional groups of phytochemicals present in the CsTe-extracts that are responsible for the stabilization and/or reduction of the CsTe-AgNPs (Dube *et al.,* 2020). The FTIR spectra of CsTe-extract and CsTe-AgNPs at pH 6 (**Figure 4.10A**) exhibited intense absorption bands at 3367.89 and 3319.26 cm^{-1} which are assigned to the -O-H group which represents the presence of alcohol and phenols. The bands at 2932.42 and 2922.67 cm^{-1} are assigned to the -C-H group indicating the presence of alkanes. The bands at 1862.44 and 1807.60 cm^{-1} are assigned to -C=C- group which indicates the presence of aldehydes; while bands at 1058.90 and 1067.80 cm^{-1} assigned for -C-N- stretching vibrations

indicating aromatic amines that are involved in the reduction and capping of CsTe-AgNPs at pH 6. The FTIR spectra of CsTe-extract and CsTe-AgNPs at pH 11 (**Figure 4.10B**) indicated bands at 3413.79 and 3437.26 cm^{-1} assigned to the -N-H group which indicates the presence of amines. The bands at 2123.27 and 2061.19 cm^{-1} are assigned to -C=C- which indicates the presence of aldehydes. The bands at 1613.90 and 1628.42 cm^{-1} show N-H group which indicates the presence of amines. While the bands at 1073.92 and 1068.82 cm^{-1} are assigned for -C-N- which indicates the presence of aromatic amines. Comparison of the CsTe extract and CsTe-AgNPs illustrated that while the extract and biosynthesized AgNPs had bands with similar widths, shifts in the absorption bands of the NPs, indicating the presence of amino acids, proteins, and flavonoids on the surface of the CsTe-AgNPs (**Table 4.2**).

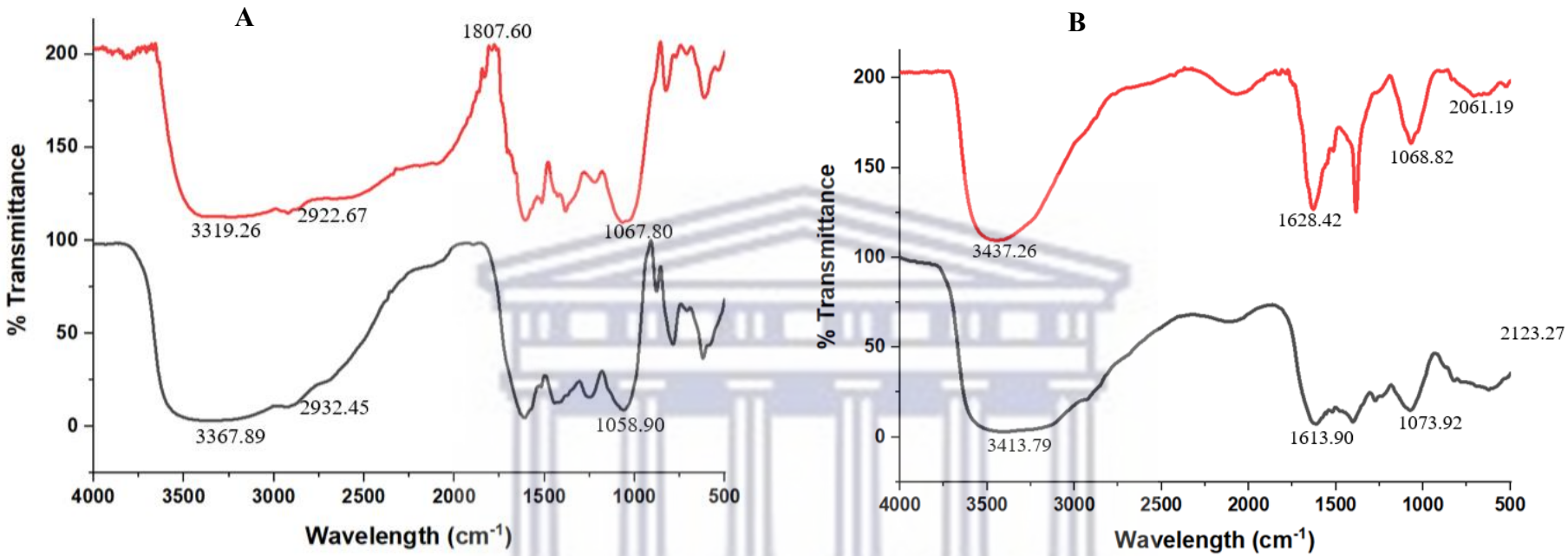


Figure 4.10: FTIR analysis of CsTe-extracts in comparison with CsTe-AgNPs. A-CsTe-AgNPs at pH 6. B- CsTe-AgNPs at pH 11.

Table 4. 2: Shifts of the FTIR spectra bands (cm^{-1}) of major peaks of CsTe extracts and AgNPs.

CsTe-Extracts pH 6	CsTe-AgNPs pH 6	Shift values	Functional group	CsTe-Extracts pH 11	CsTe-AgNPs pH 11	Shift values	Functional groups
3367.89	3319.26	+48.63	O-H	3413.79	3437.26	-23.47	N-H
2932.42	2922.67	+0.75	C-H	2123.27	2061.19	+62.08	-C=C-
1862.44	1807.60	+54,84	-C=C-	1613.90	1628.42	-14.52	N-H
1058.90	1067.80	-8.9	C-N	1073.92	1068.82	+5.1	C-N

4.4 Stability test of CsTe-AgNPs

In various biological media, stable NPs do not clump together or aggregate; rather, they stay dispersed throughout the medium (Dube *et al.,* 2020). The stability analysis was performed from 24 - 72 hrs at 37 °C since majority of the *in-vitro* and *in-vivo* applications are performed at that respective temperature. Various biological media including MHB, PDB, DPBS, DMEM As seen in **Figure 4.11,** CsTe-AgNPs were stable in all the media used, however SPR peak of CsTe-AgNPs were seen to flatten over time all media used. The high protein content of the MHB may have caused the absorption peak to flatten upon with time, negatively affecting the colloidal stability and causing agglomeration (Tyavambiza *et al.,* 2021).

Initially, CsTe-AgNPs had an SPR peak at 420 nm, however after 48 hours redshift in the SPR peak was observed at 430 nm possibly indicating the formation of AgNPs-protein corona complex. AgNPs bound to proteins exhibit an increase in wavelength, indicating that a protein layer has developed on their surface (Tomak *et al.,* 2022). DMEM complete and incomplete medium also displayed flattening tendency over time. It is possible that the variation in the pH of DMEM might have affected AgNPs stability by affecting the surface change of NPs possibly leading to aggregation. The CsTe-AgNPs were highly stable without any aggregation for 72 hrs in PDB. However, broader SPR peak were observed with increase in time possibly suggesting anisotropic behaviour of NPs.

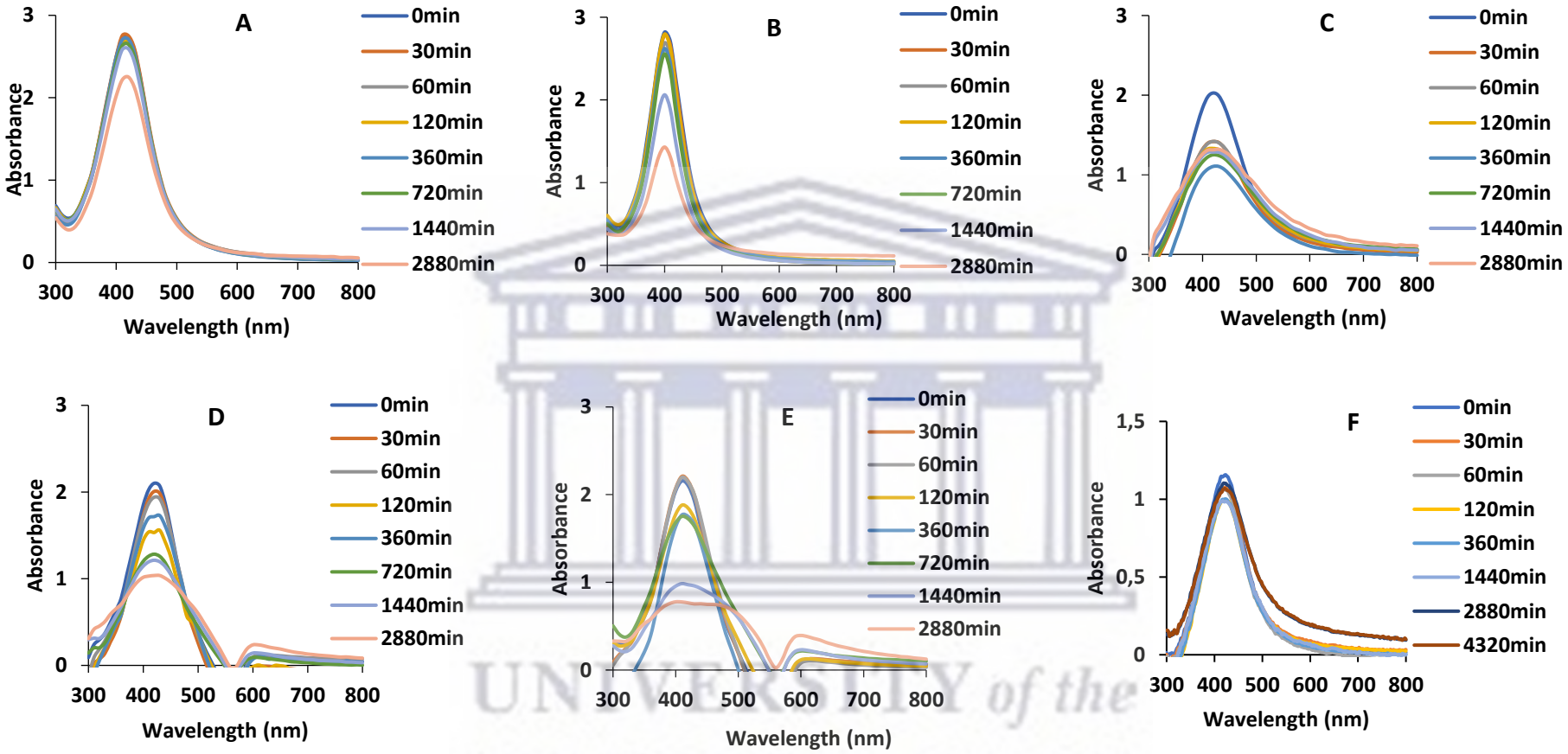


Figure 4.9: Stability test of CsTe-AgNPs in various biological media. A-ddH2O, B-DPBS, C-MHB, D-DMEM C, E-DMEM NC, F-PDB.

4.5 Antimicrobial activity

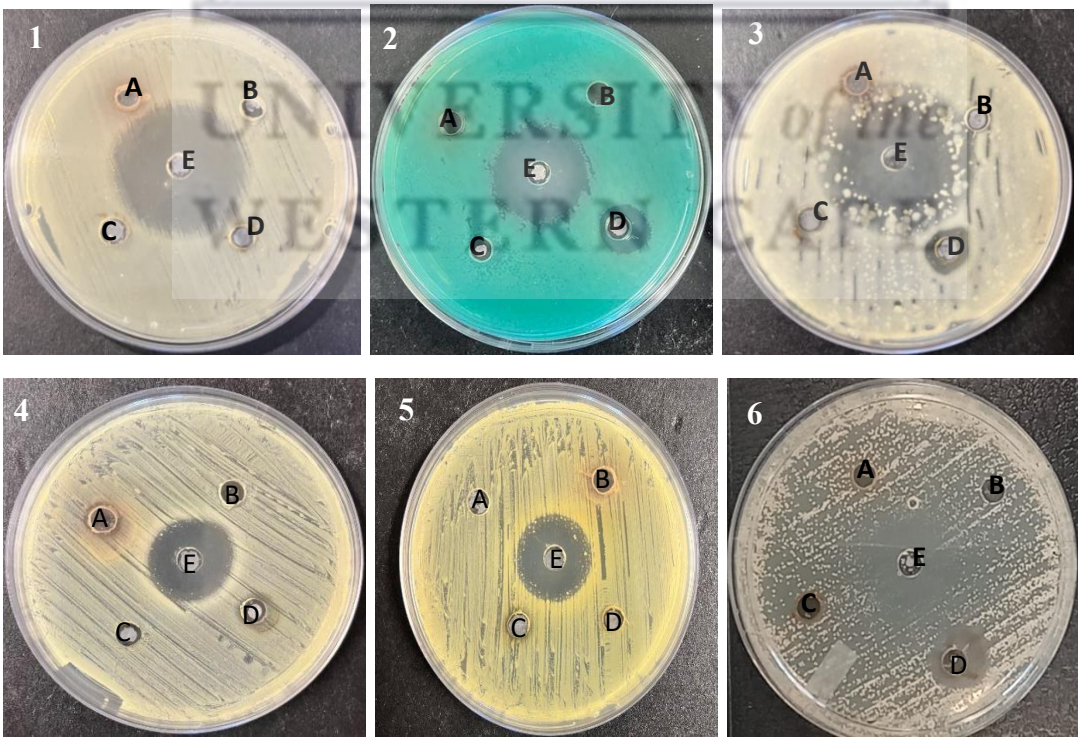
The CsTe-AgNPs were tested for their antimicrobial activity against several skin pathogens, including Gram-negative, Gram-positive bacterium as well as fungal strains, namely *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *MRSA*, and *C. albicans* using agar well diffusion assay. The agar well diffusion method was used to assess the cultures for the formation of antimicrobial metabolites (Sethi *et al.,* 2013). Zones of inhibition were used as an indication for the susceptibility of NPs against the pathogens tested. As shown in **Figure 4.12**, different treatments were used including CsTe-AgNPs at pH 6 and pH 11 (23 µg/mL), CsTe-extracts (12.5 mg/mL), positive control ciprofloxacin for bacterium (10 – 15 µg/mL) and cycloheximide for fungal strains (25 mg/mL), as well as negative control (MHB and PDB) against different pathogens. As expected, all tested strains were susceptible to the effects of the positive control, as showed in **Table 4.3**.

The CsTe-extracts (pH 6) slightly inhibited the growth of *K. pneumoniae* with zones of inhibition at 7 ± 0 mm, in contrast the CsTe-AgNPs at pH 6 had no effect on any of the tested strains. Possible reason which may have led to the CsTe-extracts to have activity to *K. pneumoniae* may be because the pathogen is more susceptible to the bioactive compounds in the extract compared to other bacterial strains. These plants include the diverse mixtures of bioactive compounds. Therefore, the observed activity against *K.pneumoniae* may be attributed to the synergistic action of a complex mixture of compounds with antimicrobial activity, as stated in numerous scientific papers (Peker and Ulusoy, 2021, Taweechaisupapong *et al.,* 2006, Inácio *et al.,* 2013). The other possible factor might be the concentration of extracts and NPs used. The concentration of antimicrobial agents in the extracts was higher than that of the NPs, resulting in increased antimicrobial efficacy against the targeted microorganisms (Chipinga, 2018, Niken-Dharmayanti *et al.,* 2020, Uezato *et al.,* 2004).

Although, no activity was observed for the CsTe-AgNPs at pH 6. The CsTe-AgNPs at pH 11 inhibited various Gram-negative bacterium (*P. aeruginosa* and *K. pneumoniae*) and Gram-positive fungal strain (*C. albicans*). *P. aeruginosa* had zones of inhibition of 10 ± 0 mm, followed by *K. pneumoniae* with zones of 8 ± 0 mm inhibition at and lastly *C. albicans* with the least zone of inhibition of 7 ± 0 mm. This observed differential bactericidal/fungicidal

activity of the CsTe-AgNPs at pH 11 might be due to small size of AgNPs (~7 ± 3.849 nm). The smaller particle size has large surface area, allowing for more frequent interaction with microorganisms, by completely destroying or effectively inhibiting their growth (Petrenko *et al.,* 2020, Qasim *et al.,* 2018, Sharmin *et al.,* 2021, Asadi, 2014). Several studies have demonstrated that Gram-negative bacteria are generally affected by AgNPs with sizes ranging from 1 to 10 nm by interfering with DNA, which causes AgNPs bactericidal effects (More *et al.,* 2023, Morones *et al.,* 2005).

This findings might be due to the fact that Gram-negative bacteria have a thinner cell wall, more lipopolysaccharides, and fewer peptidoglycan layers than Gram-positive bacteria, which makes them more susceptible to AgNPs (Sobi *et al.,* 2022, Shahi *et al.,* 2018, Petrenko *et al.,* 2020). So, AgNPs can easily pass through the cell wall thinner layer and interact with lipopolysaccharides and other parts, making the cell membrane softer and disrupting normal cellular processes (Al-Rufaie and AL-Zubaidi, 2018, Ghotaslou *et al.,* 2017). No zones of inhibition were observed against all Gram-positive bacterial strains. This might be due to their structure having more peptidoglycan layers thus hindering the NPs entry.



Key: **A-** Extract

B- Negative control (MHB/PDB)

C- CsTe-AgNPs at pH 6

D- CsTe-AgNPs at pH 11

E- Positive control (ciprofloxacin/cycloheximide)

Figure 4.12: Antibacterial activity using CsTe-AgNPs against bacterial/fungal strains. (1). *E. coli*, (2). *P. aeruginosa*, (3). *K. pneumoniae*, (4). *S. aureus*, (5). *MRSA*, (6). *C. albicans.*

Table 4. 3: Representing zone of inhibition for bacteria treated with CsTe-AgNPs, ciprofloxacin/ciprofloxacin and extracts.

Microbial strains	Ciprofloxacin/cyclohexi mide (ZOI mm)	CsTe-AgNPs pH 6 (ZOI mm)	CsTe-AgNPs pH 11 (ZOI mm)	CsTe extra (ZOI mm)
E. coli	28 ± 0	0 ± 0	0 ± 0	0 ± 0
S. aureus	18 ± 0	0 ± 0	0 ± 0	0 ± 0
MRSA	20 ± 0	0 ± 0	0 ± 0	0 ± 0
C. albicans	11 ± 0	0 ± 0	7 ± 0	0 ± 0
P. aeruginosa	25 ± 0	0 ± 0	10 ± 0	0 ± 0
K. pneumoniae	26 ± 0	0 ± 0	8 ± 0	7 ± 0

4.5.1 Antibacterial activity using microbroth dilution assay.

The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial agent that effectively hinders the growth of a microorganism within a time frame of 18 to 24 hrs (Javed *et al.,* 2015). The MIC of CsTe-AgNPs and CsTe-extracts were

determined against several Gram-negative bacterial and fungal strains that previously showed activity as determined by agar well diffusion assay (**Figure 4.12**). As seen **in Figure 4.13,** the MIC values for *K. pneumoniae* and *P. aeruginosa* was found to be 12.5 ± 0 µg/mL and 12.5 ± 5.893 µg/mL, respectively. Whereas for *C. albicans* MIC was found to be slightly higher at 25 ± 5.449 µg/mL.

The Alamar blue dye was used for the visual representation of the MIC results. The Alamar blue assay relies on the enzymatic conversion of resazurin to resorufin by viable cells exhibiting active metabolic activity (Bonnier *et al.,* 2015, Kiilll *et al.,* 2017). The conversion occurs due to the presence of a reducing enzymes inside living cells, resulting in the formation of a pink, fluorescent compound (Zlatskiy *et al.,* 2020, Kiilll *et al.,* 2017). However, sometimes in the case of signal plateau, errors, or longer incubation with the dye, the pink, fluorescent compound might reach to a saturation point, where in the solution remains colourless. This signifies that the maximum level of resazurin reduction has been attained, and additional incubation will not provide any further insights into cellular metabolism or viability (Boedicker *et al.,* 2008, Gong *et al.,* 2020). As seen in **Figure 4.13**, the MIC of pathogens were represented by a blue colour, showing the inhibition at various concentrations. However, at lower treatments concentration with the CsTe-AgNPs a pink colour is observed for the bacterial stain and a colourless colour is observed for the fungal strain. It is possible that the alteration of the dye's colour may be attributed to certain components present in the fungal species or the culture medium, which could potentially induce a chemical reaction (Gulzar *et al.,* 2020).

The minimum bactericidal/fungicidal concentration (MBC/MFC) of CsTe-AgNPs was also determined against various pathogens. As shown in **Figure 4.14**, the MBC of *K. pneumonia* was found at 12.5 ± 0 µg/mL; and *P. aeruginosa* had slightly higher MBC of 25 ± 5.893 µg/mL, indicating that *P. aeruginosa* is bacteriostatic at 12.5 µg/mL and bactericidal at 25 µg/mL. Whereas *C. albicans* displayed similar MIC and MFC of 25 ± 5.449 µg/mL. The CsTe-AgNPs bactericidal/fungicidal mechanism of action is still unknown; several researches have established potential NP bactericidal mechanisms, which includes adhesion to the surface of microbial membranes, penetration of AgNPs into the cells and disruption of biomolecules and intracellular damage, and induction of cellular toxicity by generating reactive oxygen species (ROS) that cause oxidative damage to a cell, thereby disrupting the signal transduction

pathways of the cells (More *et al.,* 2023, Bezza *et al.,* 2020, Bruna *et al.,* 2021). Other studies have postulated that the AgNPs bactericidal effects may be caused by their negatively charged nature, which can attack Gram-negative bacteria by metal depletion (Fayaz *et al.*, 2010).

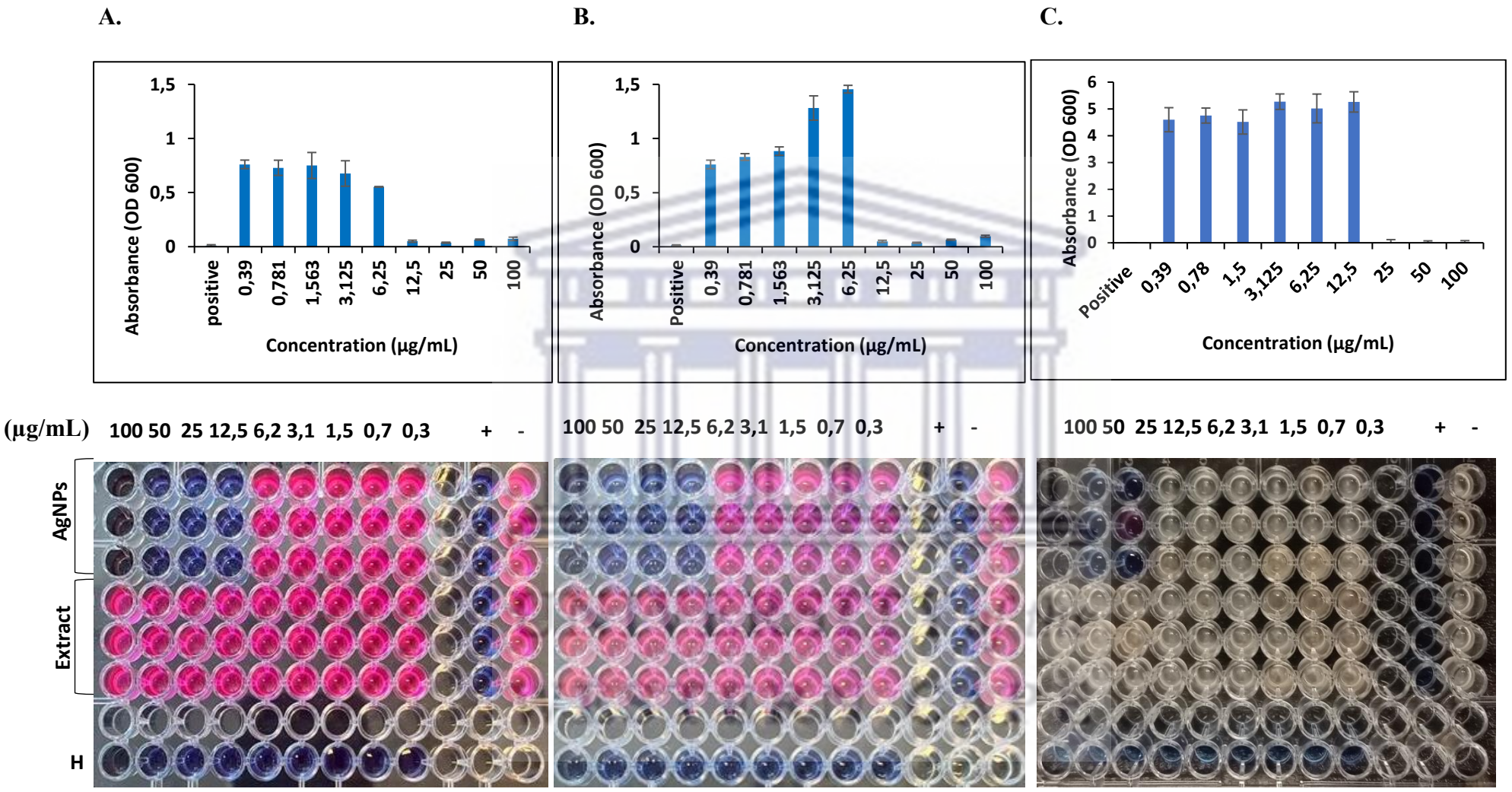
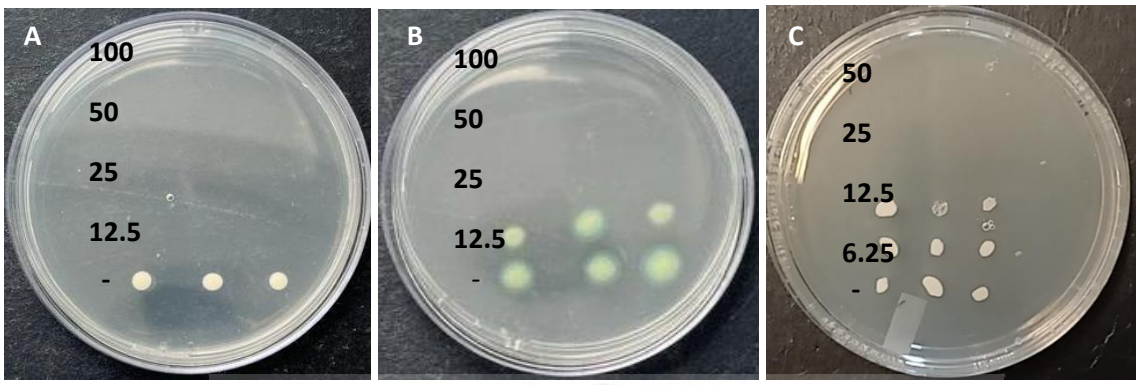


Figure 4.13: Minimum inhibitory concentration of CsTe-AgNPs A. K. pneumoniae, B. P. aeruginosa and C. C. albicans



K. pneumoniae ***P. aeruginosa*** ***C. albicans***

Figure 4.14: Minimum bactericidal/fungicidal concentration of CsTe-AgNPs. A. K. pneumoniae, B. P. aeruginosa and C. C. albicans.

4.6 Antioxidant activity of CsTe-AgNPs

The antioxidant activity of the CsTe-extract and CsTe-AgNPs was assessed using DPPH assays, a widely used method for evaluating antioxidant activity. This assay, known as the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay, is a technique used to measure the antioxidant activity of a substance. The principle underlaying this technique involves the scavenging of DPPH radicals by antioxidants through proton donation, leading to the formation of reduced DPPH, which can be quantified by monitoring the decrease in absorbance at a wavelength of 517 nm (Naganathan and Thirunavukkarasu, 2017). When a DPPH solution is mixed with a solution of a substance capable of donating a hydrogen atom, this violet colour disappears, resulting in the light-yellow reduced form of the DPPH radical (DPPH-H) (Gulcin and Alwasel, 2023).

Free radicals are unstable molecules characterised by free unpaired electrons, making them highly reactive and attempt to bond with other electrons and form covalent pairs. The free

radicals are responsible for initiating various diseases like Parkinson disease, neural disorder, mild cognitive impairment, and aging(Guntur *et al.,* 2018). Antioxidants control oxidative reactions, either by inhibiting, delaying, or hindering the oxidation of the biomolecules (Sies, 1997, Kumar *et al.,* 2011). Therefore, antioxidant plays a critical role in reducing the oxidation damage caused by reactive oxidation species (ROS) (Omomowo *et al.,* 2020).

As shown in the **Figure 4.15** below, the results of the DPPH-free radical elimination revealed that both CsTe and CsTe-AgNPs are capable of scavenging elevated concentrations of free radicals. However, CsTe-AgNPs exhibited a lower free-radical scavenging ability as compared to the CsTe aqueous extracts. It is possible that the surface of the CsTe-AgNPs may have a certain coat that may hinder the interaction between the NPs and free radicals, thus resulting in decreased scavenging activity. Furthermore, the DPPH radical scavenging activity of the CsTe, CsTe-AgNPs and ascorbic acid (used as standard), showed a dose-dependent increase. It was noticed that antioxidants ability is dependent on the concentration of the solution used. This suggests an important relationship between the concentration and antioxidant activity.

When examining the antioxidant activity at various concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL), distinct pattern was observed. The CsTe aqueous extract showed almost the same pattern of the ascorbic acid in terms of free-radical scavenging activity at concentrations of 25, 50, 100 and 200 µg/mL. However, at the highest concentration 400 µg/mL, and a lower concentration of (3.125, 6.25 and 12.5 µg/mL), ascorbic acid exhibited higher free-radical scavenging activity as compared to the CsTe aqueous extract. At 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL, the antioxidant activity of CsTe-AgNPs, CsTe and ascorbic acid where quantified at: (5, 8, 12, 21, 32, 36, 44, and 46%), (22, 36, 54, 76, 72, 80, 78, and 77%), and (50, 61, 72, 73, 74, 75, 80 and 161%), respectively. Notably, the highest antioxidant capacity of 161% at 400 µg/mL was observed for ascorbic acid free-radical scavenging activity as compared to the CsTe aqueous extract.

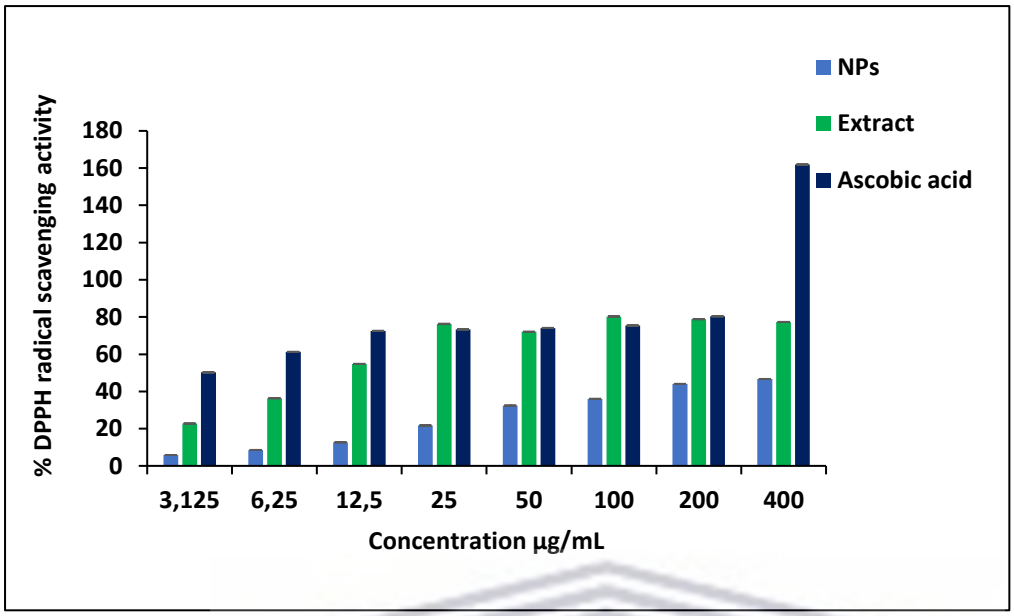


Figure 4.15: The antioxidant activity of CsTe aqueous extract and CsTe-AgNPs at different concentrations as indicated by the % scavenging activity. NPs, is the CsTe-AgNPs, extract is the CsTe-cocktail extract and ascorbic acid is the used standard.

4.7 Cytotoxicity analysis

As seen in **Figure 4.16**, the cytotoxicity analysis of CsTe-AgNPs, CsTe-extracts and allantoin were carried out, using similar concentrations (ranging from 0.78 – 50 µg/mL). Allantoin was used as a standard in this study, and it was observed to promote proliferation of the normal skin cell at all concentration used, displaying no sign of toxicity against the KMST-6 cells. In contrast, the CsTe-AgNPs showed to be slightly toxicity to the KMST-6 cells at all concentrations used (0.78 – 50 µg/mL), with a constant cell viability range between 85 - 90 %, possibly suggesting slight toxicity of NPs as compared to the negative control. Although not indicated in the Figure below, 10% DMSO was used as positive control. The CsTe-extracts displayed a dose-dependent activity to the normal skin cells. Whereas, at high concentration of the extracts (ranging from 6.25 – 50 µg/mL), no toxicity was observed as indicated by cell viability above 100 %. This finding suggests that the extracts did not harm the cells at this concentration range. However, by decreasing the concentration further (3.125 – 0.78 µg/mL) the extracts displayed slight toxicity to the normal skin cells. The overall results showed a significant difference between CsTe-AgNPs and the standard control (allantoin) ($p^{**}<0.01$). Whereas no significant difference was observed between the AgNPs and the extract. Moreover, the extract and allantoin did not show any significance difference.

The results might suggest that higher concentration of phytochemicals in the extracts induces cell proliferation. Whereas lower concentration of phytochemicals might trigger cytotoxicity to the normal skin cells. Several studies have shown that phytochemicals may promote cell proliferation and offer health benefits (Čižmárová *et al.,* 2023, Garutti *et al.,* 2022). As we compare the extracts with the AgNPs it is observed that the extracts perform better at a higher concentration. The toxicity of AgNPs might be caused by their small size and large surface area causing enhanced interactions with the cells thus leading to the toxic effect.

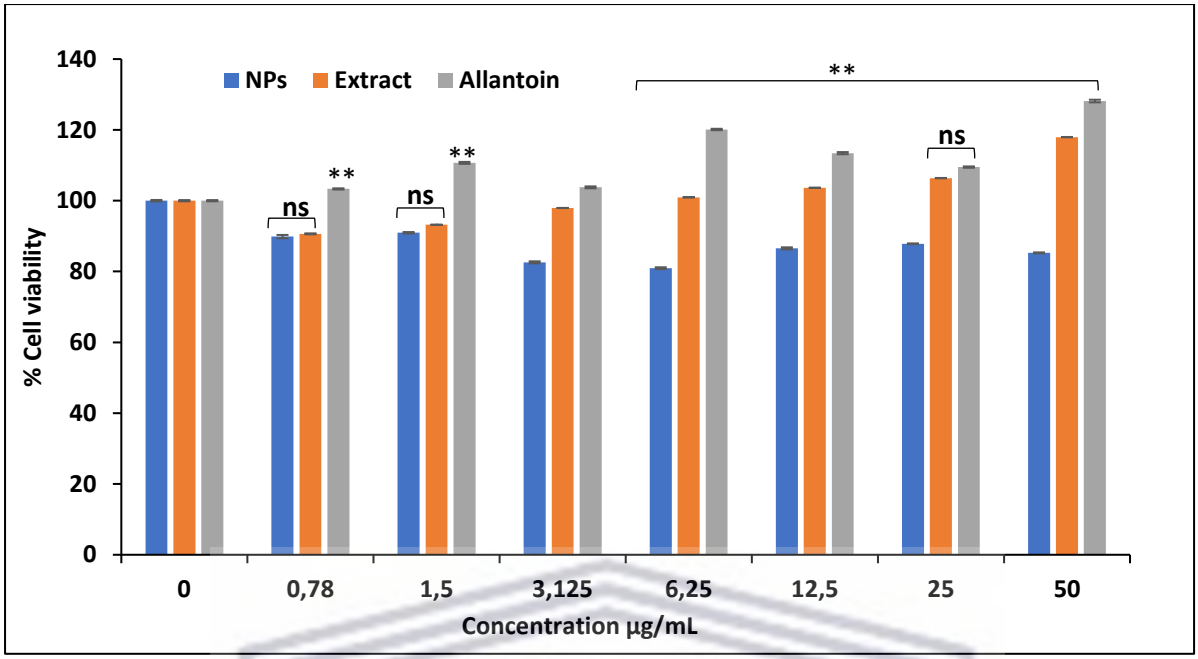


Figure 4.16: Analysis of the cytotoxic effects of CsTe-AgNPs against KMST-6 cell line.

The level of significance is indicated by p<0.01 and p ^{ns} >0.05.**

CHAPTER 5

5. CONCLUSION

This study has demonstrated a simple, efficient, and green approach which utilizes CsTe-cocktail as a reducing and stabilizing agent in the synthesis of AgNPs. CsTe-AgNPs were successfully synthesized at the normal plant cocktail pH (6) and elevated pH (11). The characterization results confirmed the formation of uniform, and stable AgNPs. The CsTe-AgNPs displayed antimicrobial activity against two bacterium and fungal strains (*K. pneumoniae*, *P. aeruginosa* and *C. albicans*). *K. pneumoniae* and *P. aeruginosa* are one of the major pathogens that are known for their ability to gain resistance against various antimicrobial agents thus posing a challenge in the hospitals settings. Both *K. pneumoniae* and *P. aeruginosa* are responsible for healthcare-associated infections because they are dominant in hospitals and can live on surfaces and equipment. Infections caused by this bacterium have become a global challenge, which makes it difficult to successful treat infections caused by them.

However, these study findings highlight the significance of the green synthesis method as an environmentally friendly and cost-effective approach for developing potent antimicrobial agents against multidrug-resistant pathogens. The environmentally friendly nature of this method, coupled with the demonstrated antimicrobial efficacy, opens new avenues for the development of sustainable and effective antimicrobial agents. In order to combat AMR and lessen the environmental risk brought on by the overuse of antibiotics, CsTe-AgNPs may be successfully used in the pharmaceutical industry. The antioxidant properties of CsTe-AgNPs were also successfully investigated using DPPH. The cytotoxicity of CsTe-AgNPs was evaluated using MTT assay against normal skin cells (KMST-6). The results displayed slight toxicity of CsTe-AgNPs at all used concentrations (0.78 – 50 µg/mL) with a constant cell viability (85 - 90 %). Whereas CsTe-cocktail displayed to promote cell proliferation at higher concentration than at a lower concentration. Having a dose-dependent response to the normal skin cell.

5.1 Limitations

One major limitation of this work was the limited infrastructure to carry out research on antifungals study. This limitation could potentially impact the results due to the prolonger incubation times and difficulties in maintaining constant temperature control, these challenges

may have an effect on the results and might raise the possibility of microbial culture contamination. Notably, the widespread power and energy limitations in the country are a significant general problem that may have an impact on the conditions in which skin cells were used in the studies. These difficulties highlight the significance of identifying and resolving resource and environmental related constraints that may add complexity and variability to the research findings.

5.2 Future Recommendation

Future research should focus on the inclusion of CsTe-AgNPs in bandages and might also explore their potential synergistic effects with existing antibiotics. Even though CsTe-AgNPs appeared to have to good antimicrobial activity against various strains, more study is required to investigate the cytotoxicity of CsTe-AgNPs against various skin cells types including HaCaT cell line. It is possible that this CsTe-AgNPs might be slightly hurmful to certain types of normal skin cells, mainly due to differences in cell physiology, uptake mechanism and cellular responses. Future studies may focus on the investigating the toxicity of CsTe-AgNPs and explore alternative measures to minimise the cytotoxicity of CsTe-AgNPs. Also the possibility of exploring this CsTe-AgNPs *in vivo* studies might be necessary prior to their possible application in pharmaceuticals industies. In additional, since CsTe aquous extract displayed less toxicity to the normal skin when used at higher concentrations, the potential mechanism by which phytochemicals use to promote cell proliferation and must also be explored and how they can be used in improving wound healing might also be investigated.

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