GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *MORINGA OLEIFERA* EXTRACTS AND THEIR EVALUATION AGAINST ANTIBIOTIC RESISTANT STRAINS OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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Abstract

It is of great concern that bacteria are forming resistance to common antibiotics meant to prevent or cure them. For this reason, researchers are engaged in synthesis of nanoparticles using medicinal plants to solve the problem of antibiotic resistance in bacteria. The aim of the study was to synthesize silver nanoparticles (AgNPs) using *M. oleifera* extracts and compare antibacterial activities of AgNPs prepared in *M. oleifera* extracts with AgNPs of common antibiotics (penicillin G and streptomycin) against antibiotic resistant strains of *Escherichia coli* and *Staphylococcus aureus*. Synthesized AgNPs were characterized using UV-vis spectroscopy, fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Disc diffusion method was used for initial screening of antibacterial activities and their minimum inhibitory concentration (MIC) was determined. Formation of AgNPs was confirmed by colour change from pale yellow to dark brown-black. UV-vis spectra analysis showed maximum peak of 400-440 nm. FTIR analysis revealed the presence of hydroxyl and carbonyl functional groups, as well as C-N and C-O bonds that might be responsible for capping, stabilizing and reducing AgNPs. SEM analysis revealed crystalline AgNPs with mean average size of 46.49 ± 0.73 nm to 60.96 ± 1.17 nm. The combination of AgNPs with antibiotics showed better antibacterial activities as compared to AgNPs alone and streptomycin alone. Lowest MIC values were 12.5 mg/mL, 1.56 mg/mL and 3.13 mg/mL for Mo-AgNPs, Mo-AgNPs-Penicillin G and Mo-AgNPs-streptomycin combination respectively. It can then be concluded that *M. oleifera* has strong potential for synthesis of AgNPs, and combination of AgNPs with common antibiotics sheds light in the direction of decreasing odds of antibiotic resistance development of these pathogens.

Keywords: Antibacterial activity, FTIR, Green synthesis, *Moringa oleifera*, SEM, UV-vis.

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List of abbreviations and/ or acronyms

AgNPs- Silver nanoparticles.

$AgNO_3$- Silver nitrate.

Ag^+- Silver ion.

M. oleifera- *Moringa oleifera.*

SEM- Scanning Electron Microscopy.

FTIR- Fourier Transform Infrared Spectroscopy.

Mo-AgNPs- *Moringa oleifera* extract mediated silver nanoparticles.

Mo-AgNPs-Penicillin- Combination of *Moringa oleifera* extract mediated silver nanoparticles and penicillin G.

Mo-AgNPs-Streptomycin- Combination of *Moringa oleifera* extract mediated silver nanoparticles and streptomycin.

ZOI- Zone of inhibition.

MIC- Minimum inhibitory concentration.

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Declaration

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1. Introduction

1.1 Background of the study

The world is facing an increasing problem in antibiotic resistant strains of bacteria. Some bacteria have begun to show resistance to third generation of antibiotics (WHO, 2014). For this reason, several efforts are being made by researchers worldwide to find new antibacterial agents. The application of nanotechnology is rapidly growing in pharmaceutical and biomedical fields, aiming to overcome bacterial resistance (Morones *et al*., 2005). Nanotechnology is a field of science that manipulates matter on an atomic, molecular and supramolecular level with the aim of creating nanostructures with improved functionalities (Moodley *et al*., 2018). Nanoparticles are particles of less than 1 micrometer (µm), usually 1-100 nanometer (nm). They have enhanced properties and a wide scope of diverse applications based on their features such as morphology, size, shape and distribution (Bhusnure *et al*., 2017).

Nanoparticles can be synthesized using chemical, physical or biological (green synthesis) methods. However, silver nanoparticles (AgNPs) are preferentially synthesized using biological than chemical and physical methods because biological methods are environmentally friendly. Chemical methods produce nanoparticles that are toxic to the host's normal cells and lead to non-ecofriendly by-products which may disturb normal cells of the host (Das *et al*., 2017). Among other metal nanoparticles, silver is gaining popularity because it exhibits new and improved properties; it is a metal known for its broad-spectrum of antimicrobial activities against gram positive and gram negative bacteria, fungi, protozoa and certain viruses (Hendiani *et al*., 2015). For instance, AgNPs have been found to possess

antibacterial activities against *Escherichia coli, Staphylococcus aureus, Vibrio cholera, Pseudomonas aeruginosa* and *Salmonella typhi* (Li *et al*., 2010). According to Nahar *et al.*, (2020), there are two groups of nanoparticles, organic and inorganic nanoparticles. Organic nanoparticles are carbon nanoparticles while inorganic nanoparticles are noble (gold and silver), magnetic and semiconductor nanoparticles.

Moringa oleifera Lam (*M. oleifera*), commonly known as drumstick tree, belongs to the family *Moringaceae*. *M. oleifera* is used as an ingredient in Indian diet, however, it is also found in several other areas such as Africa and America where fruits and leaves are used as vegetables (Ghosh *et al*., 2014). In addition to nutritional values, *Moringa* also possess therapeutic activities. It has been shown that extracts of the plant have high antioxidants and antimicrobial activities against gram positive and gram negative bacteria. The leaves have been used for medical purposes such as treating bronchitis, eye diseases and further used as an antitumor and wound healing activities (Moodley *et al*., 2018).

Studies have shown that combining nanoparticles with antibiotics reduce the toxicity of both agents towards human cells by decreasing the requirement for high dosage and enhances their antimicrobial activities (Hassan *et al*., 2016). A combination of AgNPs and common antibiotics showed better antimicrobial properties against microorganisms than when they are applied alone. Antibiotics when combined with nanoparticles restore their ability to destroy bacteria that have acquired resistance to them. This is because, nanoparticles labelled with antibiotics increase the concentration of antibiotics at the site of bacterium–antibiotic interaction and facilitate the binding of antibiotics to bacteria (Hassan *et al*., 2016). Therefore, researchers are using nanoparticles in general, and silver nanoparticles in

particular, and in combination with antibiotics to solve the problem of emergence of multi-drug resistant bacteria (Rai *et al*., 2012).

The present study evaluated synergistic effects of AgNPs prepared through the interaction of two common antibiotics and *M. oleifera* extracts against antibiotic resistant strains of *E. coli* and *S. aureus*. The two common antibiotics studied in combinations with AgNPs were penicillin G and streptomycin.

1.2 Statement of the problem

To the researcher's knowledge, evaluation of antibacterial activities of AgNPs prepared in Namibian *M. oleifera* extracts and in combination with antibiotics against antibiotic resistant strains of *E. coli* and *S. aureus* have not been documented yet. This may present a limit in the discovery of new antibacterial agents with potential of overcoming bacterial resistance.

1.3 Objectives of the study

a) To synthesize AgNPs using leaf, seed and root extracts of *M. oleifera.*
b) To compare antibacterial activities of silver nanoparticles prepared in *M. oleifera* extracts with silver nanoparticles of selected common antibiotics against antibiotic resistant strains of *E. coli* and *S. aureus*.

1.4 Hypothesis of the study

H_O: As prepared AgNPs using *M. oleifera* extracts and in combination with common antibiotics do not have synergistic antibacterial activities against antibiotic resistant strains of *E. coli* and *S. aureus.*

H_A: As prepared AgNPs using *M. oleifera* extracts and in combination with common antibiotics have synergistic antibacterial activities against antibiotic resistant strains of *E. coli and S. aureus.*

1.5 Significance of the study

Antibiotic resistance of pathogenic bacteria has become a major problem worldwide. With the ever-increasing threat of antibiotic resistance by strains of bacteria, this research provides alternative therapy or new antibacterial agents. Furthermore, the study presents a non-toxic as well as eco-friendly procedure for synthesizing silver nanoparticles. This technique offers a simple and efficient way for synthesis of nanoparticles with good antibacterial properties. Taking all these into consideration, this study has potential to contribute to existing knowledge on antibacterial agents that may be used to treat antibiotic resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), therefore the study can shed light on new pharmaceutical agents.

1.6 Limitations of the study

Biosynthesis of AgNPs is accompanied by many factors that may influence the quality of the results. At nanoscale range, the particle size leads to large surface area per mass where a large number of atoms are in immediate contact and available for reaction. This size, morphology, stability and properties (chemical and physical) of the metal nanoparticles are strongly influenced by the experimental conditions. Hence, the design of a synthesis method in which size, morphology, stability and properties are controlled, has also become a thrust area of research.

1.7 Delimitations of the study

M. oleifera was collected in Windhoek, Khomas region, Namibia and biological analysis was performed on this plant. Only *E. coli* and *S. aureus* was used in this study. This may make it difficult to generalize results to plants in other different regions as well as other bacterial strains that were not used in this study.

2. Literature review

2.1 Antibacterial activities of *M. oleifera* extracts

Several studies have reported on antibacterial activities of *M. oleifera* extracts using different solvents and parts of the plant. For instance, Devi *et al*., (2011) found that among the leaves, bark, seeds and flesh (fruits) extracts of *M. oleifera*, flesh extracts had uppermost activities against all test organisms (*S. aureus, E. coli, Bacillus subtilis, Klebsiella pneumoniae, Shigella dysentriae*) while other parts were only effective against some bacterial strains. However, methanolic extracts of bark showed highest inhibition zone (22 mm) against *B. subtilis*. Othman and Ahmed (2017) compared leaf ethanol extracts of *M. oleifera* and *Camellia sinensis* against multidrug resistant strains of *E. coli* and *S. aureus.* In the study, it was found that *M. oleifera* possess better antibacterial activities than *C. sinensis*, however, both plants had an MIC that ranged between 10-20 mg/mL.

Bello and Jamiu (2017) found that among ethanol, methanol and aqueous seed extracts of *M. oleifera*, methanol extracts have better activities where it was showed that *Pseudomonas aeruginosa* is the most susceptible (20 mm) followed by *E. coli* (18 mm) and lastly *S. aureus* (15 mm). The study also provided information that antibacterial activities increase with increasing concentration of extracts. In the study done by Ervianingsih *et al*., (2019) results

showed that leaf ethanol extracts of *Moringa* have antimicrobial activities against *Staphylococcus epidermis*.

Al husnan and Alkahtani (2016) evaluated the impacts of *Moringa* aqueous extracts on pathogenic bacteria and fungi. It was found that the impact is greater on bacterial strains as compared to fungal species. Eight bacterial strains were used (*Bacillus cereus, Klesiella pneumonia, Salmonella typhi, Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa* and *Proteus vulgaris*). Among the strains, *S. typhi* was the most sensitive with an inhibition zone of 23.5 ± 0.45 mm and least inhibited being *P. aeruginosa* with 12.5 ± 0.5 mm inhibition zone. With Minimum inhibitory concentration however, results showed that *B. cereus* needed the lowest concentration (0.652 mg/mL) to be inhibited while *P. aeruginosa* showed to be the least sensitive strain with 5.265 mg/mL of MIC.

Garga *et al*., (2019) compared the synergistic activities of leaves and seeds of *M. oleifera* extracts on *S. aureus,* using methanol and ethyl acetate. Their results revealed that both leaves and seeds have antibacterial activities on test bacterium strain. Methanolic extracts had improved activities than ethyl acetate using both plant materials. It was further shown that the efficiency of the extracts on bacterial growth increases with increasing concentration. Teshome *et al*., (2021) showed that chloroform, acetone and petroleum ether root extracts of *Moringa stenopetala* have growth inhibitory activities of *Salmonella thyphimurium, P. aeruginosa, S. aureus* and *E. coli. S. aureus* was the most sensitive and acetone showed superior inhibitory activities that petroleum ether and chloroform extracts.

In the study done by Singh and Tafida (2014), it was also reported that antibacterial activities of *Moringa oleifera* leaves extracts increases as concentration increases. Ethanol, methanol

and aqueous solvents were used in their study and they reported that ethanol have superior activities than methanol and aqueous extracts, with *P. aeruginosa* being most sensitive than *S. aureus* and *E. coli.* Elangovan *et al*., (2014) tested the effects of chloroform, petroleum ether and aqueous extracts of *M. oleifera* leaves on *Enterococcus faecalis, Bacillus subtilis, E. coli, S. typhi,* and *S. aureus*. It was reported that *S. aureus* is the most sensitive, with petroleum ether having superior activities on test organisms than chloroform and aqueous extracts.

Zaffer *et al*., (2014) reported that among chloroform, ethyl acetate, methanol and aqueous bark extracts of *M. oleifera*, ethyl acetate extracts have upper most antibacterial activities against test bacteria (*Staphylococcus aureus, Citrobacter freundii, Pseudomonas fluorescence* and *Bacillus megaterium*) followed by chloroform, methanol and aqueous extracts in their descending order. Among test strains, it was reported that *S. aureus* is the most sensitive. A concentration dependent inhibitory activity pattern was also reported in the study. *Staphylococcus epidermis* was tested against ethanolic extracts of *M. oleifera* leaves in the study done by Ervianingsih *et al*., (2019) in which results showed that the test bacterium strain is sensitive to the extracts with maximum inhibition zone of 14 mm. However, the positive control (tetracycline) possesses improved antimicrobial activities of 30 mm on average.

Isitua *et al*., (2016) determined susceptibility of some enteric human pathogens associated with diarrheal diseases namely; *Streptococcus pyogenes, Shigella sonnei, Shigella dysenteriae, Shigella flexneri, Shigella boydii, Proteus mirabilis, S. aureus, B. cereus, E. coli* and *P. aeruginosa.* Minimum inhibitory concentration (MIC) and disk diffusion method of ethanol and aqueous extracts of leaves were determined against ten strains. Among test

organisms, *S. dysenteriae* was highly susceptible with an MIC of 64 µg/mL and 32 µg/mL for aqueous and ethanol extracts respectively. *Shegella flexneri* was the least susceptible. Disk diffusion method showed gram positive organisms to be more sensitive to the extracts than gram negative, with *S. aureus* being most susceptible.

Amabye and Tadesse (2016) evaluated antibacterial activities of *Moringa oleifera* ethanolic and aqueous leaves extracts against *P. aeruginosa, Proteus vulgaris, Streptococcus mutans, B. subtilus, E. coli, P. aeruginosa, S. aureus* and *S. epidermidis.* Similar to previous literature, it was found that on average, ethanol extracts are more efficient that aqueous extracts. However, aqueous extracts had maximum inhibitory activities against *Proteus vulgaris* while ethanol extract was most effective against *Streptococcus mutans*. Sopandani *et al*., (2020) evaluated the effects of *M. oleifera* leaf extracts on *Enterococcus faecalis* using an irrigation method to remove it from root canal of premolar teeth. Using different concentrations of 100%, 75%, 50% and 25% of extracts, the study found that the number of *E. faecalis* in root canal decreases with increase in concentration of extract solution. In addition, it was reported that there is a statistically significant difference in all concentrations in reducing the bacteria compared to the negative control of phosphate-buffered saline. Concentrations of 75% and 100% were as effective as 5.25% NaOCl which was used as a positive control.

Shailemo *et al*., (2016) compared the antibacterial activities of *Moringa ovalifolia* and *M. oleifera* methanol, n-hexane and water seeds and bark extracts against *B. cereus, E. coli* and *E. faecalis.* In the study, it was reported that *M. oleifera* extracts have better antibacterial activities than extracts of *M. ovalifolia* and deduced that seed extracts of *M. oleifera* had broader spectrum of antibacterial activities than that of *M. ovalifolia* against three bacteria

strains. Similarly, *M. oleifera* presented lower MIC values than *M. ovalifolia.* Seed methanol and n-hexane extracts of *M. oleifera* showed an MIC of 20 mg/mL against the bacterial strains. In the case of *M. ovalifolia* seed methanol extracts, *B. cereus* presented an MIC of 50 mg/mL, 20 mg/mL for *E. coli* and *E. faecalis* was resistant. On the other hand, *M. ovalifolia* n-hexane seed extracts obtained an MIC of 50 mg/mL for *E. faecalis* while *E. coli* and *B. cereus* showed some resistance.

2.2 Phytochemical constituents and bioactive compounds of *Moringa oleifera*

Several reports in literature have reported that *M. oleifera* Lam contains bioactive compounds that aid in the plant's biomedical applications. For instance, Udofia *et al*., (2020) reported that in leaf extracts of *M. oleifera*; phenols, alkaloids, tannins and glycosides are highly abundant whereas flavonoids and saponins are moderately present, these compounds aid in inhibitory effects of the plant. Srivastava *et al*., (2020) documented that leaves contain quercetin and chlorogenic acid that plays a role in anti-diabetic activities of the plant. In addition, they have glucosinolates, isothiocyanates and β-carotene that aid in anti-inflammatory activities. Polyphenol, gallic acid, quercetin, kaempferol and glycosides present in the leaves have potential anti-cancer activities (Charoensin, 2014).

The study done by Raj *et al*., (2011) reported that roots of *M. oleifera* contains compounds such alkaloids, flavonoids, tannins, terpenoids, saponins and cardioglycosides that possess antimicrobial activities. According to Wang *et al*., (2016), seeds of *Moringa* have 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, methyl N-4-(α-L-rhamnopyranosyloxy) benzyl carbamate, and 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzylthiocarboxamide, these compounds have antibacterial activities against some pathogenic bacteria. Seeds also have a compound called lectin which has potential

antibacterial activities and because it is water soluble, it can reduce the growth of bacteria in polluted water (Wang *et al*., 2016).

In the study of Elangovan *et al*., (2014), it was found that petroleum ether and aqueous extracts of leaves have saponins, flavonoids, tannins, alkaloids and phenols. Results of Garga *et al*., (2019) revealed the presence of glycosides, alkaloids, saponins, saponin glycosides, flavonoids and cardiac glycosides in methanolic leaves extracts. Although ethyl acetate leaves extracts showed absence of some compounds, the extracts still had saponins in greater amounts compared to seed ethyl acetate extracts. Furthermore, leaves extracts had more bioactive compounds than seed extracts (Garga, *et al*., 2019). Paikra *et al*., (2017) documented that root alcoholic extracts have phytochemical constituents such as, spirachin, p-cymene, deoxy-niazimicine morginine, alpha-phellandrene whereas pods hydro-alcoholic extracts have benzyl carbamate, thiocarbamates, beta-sitosterol isothiocyanate, nitrites, thiocarbamates and undecanoate.

2.3 Nutritional composition, medicinal properties and benefits of *Moringa oleifera*

According to Gopalakrishnan *et al*., (2016), *Moringa* leaves are rich in minerals such as iron, magnesium, calcium and potassium, and they also possess vitamins (vitamin A to E). In addition, the leaves have abundant phytochemical constituents such as saponins, tannins, flavonoids and steroids. Leaves are used to treat skin diseases, ear and eye infections, asthma, hyperglycemia, pneumonia, malaria, syphilis and headaches (Rockwood *et al*., 2013). Moreover, the leaves can be used to reduce high cholesterol levels, high blood pressure and they can serve as anticancer, antidiabetic and anti-atherosclerotic agent (Jung, 2014).

Alegbeleye (2018) stated that fresh leaves of *M. oleifera* contains vitamin A, B1, B2, B3 and is a source of beta-carotene (β-carotene). The leaves can be used to treat piles, catarrh, sore throats and scurvy. Fresh leaf extracts have a wide range of biological activities like tissue protection and also possess antidyslipidemic activities, especially alcohol and aqueous extracts. Dried leaves also possess β-carotene, however, they have vitamin C and E that help in boosting the immune system and maintaining healthy skin and eyes (Alegbeleye, 2018). Vergara-Jimenez *et al*., (2017) suggested that *M. oleifera* has potential to inhibit human macrophage cytokine production that is caused by cigarette smoke and lipopolisaccharides. Reetu *et al*., (2020) stated that *Moringa* has potential for anti-diabetic properties as it was found that aqueous extracts can cure streptozotocin-induced type 1 diabetes and also insulin resistant type 2 diabetes.

Moreover, several studies suggest that *M. oleifera* can serve as an anti-cancer agent due to its ability of inducing reaction oxygen species (ROS) in cancer cells, which results in apoptosis (Reetu *et al*., 2020). Dubey *et al*., (2013) documented that *M. oleifera* has hypotensive effects due to niazinin A, niazinin B and niazimicin compounds present in the leaves of the plant. Because of these compounds, the plant has blood pressure lowering effects. Lovepreet and Jatinder (2019) reported that seeds have phytochemicals and vitamins much similar to those found in leaves, however, seeds have oleic acid and an antibiotic known as pterygospermin. Attributed to nutritional composition, seeds can be used in treating chrohn's disease and sexually transmitted infections (STI's), antiherpes-simplex virus arthritis and can be used as an anti-inflammatory agent (Rockwood *et al*., 2013).

Equally important, seeds can be used in water purification processes, powder seeds contain cationic polyelectrolytes which work as a natural flocculent to explicitize even most muddy

water. The powder joins with solids in the water and settles down, by so doing, about 90% of bacteria in the water are removed (Lovepreet and Jatinder, 2019). Seeds can be used as an appetite stimulant, diuretic and anti-flatulence agent (Meireles *et al*., 2020). A study done by Sutalangka *et al*., (2013) reported that flowers of *M. oleifera* comprises of potassium, amino acids and calcium. Therefore, *Moringa* flowers act as anti-arthritic agents and have hypocholesterolemic properties which can cure urinary diseases. Flowers can be used in clinical settings as an anti-ulcer and anti-tumor agent. They can also be used to treat swollen lymph nodes and glands of the neck and decrease serum cholesterol (Swati *et al*., 2018).

According to Adeyemi and Elebiyo (2014), roots contain minerals such as magnesium, calcium and sodium. They also have alkaloid substances like morphine and moriginine. The study further states that roots can act as anti-ulcer, anti-inflammatory agent and cardiac stimulant. According to Meireles *et al*., (2020), the roots of the plant can be use as anti-diarrheal, diuretic and ant-migraine agents. Moreover, they can be used to control dizziness, edemas, high blood pressure and gastro intestinal problems. Pods of *M. oleifera* contains carbohydrates, ash, fibre and proteins. In addition, they also contain fatty acids like oleic acid and linoleic acid. Pods can aid in treatment of diarrhea, join pains, spleen and liver problems (Gopalakrishnan *et al*., 2016; Lovepreet & Jatinder, 2019).

2.4 Antimicrobial activities of plant extract mediated AgNPs and in combination with antibiotics

In a study done by Das *et al*., (2017), results showed that a combination of silver nanoparticles and aqueous extracts of *Ocimum gratissimum* inhibits the growth of multidrug resistant strains of *E. coli* and *S. aureus* in which *E. coli* was more susceptible than *S. aureus.* Normal aqueous extracts of *M. oleifera* are effective against *Candida albicans*, however, a

combination of AgNPs and plant extracts of *M. oleifera* have greater inhibition activity against *C. albicans* (Vibhute *et al*., 2014). Ghosh *et al*., (2014) found that *Moringa* mediated silver nanoparticle leaf extracts are effective against *E. coli, S. aureus, Pseudomonas aeruginosa* and *Klebsilella pneumonia*. In their study, AgNPs exhibited more activities than streptomycin against *E. coli*.

Islam *et al*., (2021) compared antibacterial activities of crude extracts from leaves of *Moringa oleifera* and synthesized AgNPs against six bacterial strains (*Micrococcus, Vibrio cholera, S. typhi, E. coli, P. vulgaris* and *S. aureus*). It was found that using same concentration of the treatments, AgNPs have enhanced antibacterial activities than crude extracts with maximum zone of inhibition of 7.93 ± 1.68 mm and 21.67 ± 4.72 mm for crude extracts and synthesized AgNPs respectively. However, ciprofloxacin used as positive control still showed better antibacterial activities than crude extracts and silver nanoparticle, presenting maximum inhibition zone of 34.33 ± 2.08 mm.

Kalugendo and Kousalya (2019) evaluated the efficacy of silver nanoparticles of *M. oleifera* seed extracts on methicillin resistant *S. aureus* strain four and five. The results showed that strain five was more sensitive than strain four with inhibition zones of 30 mm and 5 mm respectively. Mohammed *et al*., (2020) synthesized silver nanoparticles using stem extracts of *Moringa* and tested their effectiveness against *E. coli* (ATCC 8739). In the results, it was shown that antibacterial activities increase with increasing concentration of AgNPs. 100 µg/mL produced inhibition zone of 17.5 mm while 80 µg/mL made 16.5 mm.

Arrieta *et al*., (2017) synthesized AgNPs using stem extracts of *Moringa oleifera* and evaluated antibacterial activities against *Klebsiella cloacae, S. epidermis* and *E. coli.* The growths of all strains were inhibited, with *E. coli* being most sensitive followed by *S.*

epidermis and lastly *K. cloacae* having inhibition zones of 19.5 ± 0.09 mm, 19.0 ± 0.10 mm and 18.0 ± 0.12 mm correspondingly. Brian *et al*., (2016) compared antibacterial activities of aqueous and methanol extracts of *Moringa oleifera, Murraya koingii and Ocimum sanctum* against *E. coli and S. aureus.* Among the plants, *Moringa* was found to possess uppermost antibacterial activity with highest inhibition zone of 15.1 mm for aqueous extracts and 16.25 mm for methanol extracts, all acquired for *S. aureus*. The lowest MIC value of 25 µL/mL was exhibited by the same plant against *S. aureus.*

Mofolo *et al*., (2020) evaluated anti-proliferative activities of silver nanoparticles synthesized from extracts of *Pechuel-loeschea leubnitziae* against U87 cell line. Results showed that AgNPs are cytotoxic and has potential anticancer properties. Adil *et al*., (2019) compared activities of antibiotic (ciprofloxacin) alone, Callus extract mediated AgNPs, and ciprofloxacin + extract medicated silver nanoparticles against *E. coli, Salmonella typhi, Shigella sonnei and Citrobacter amalonaticus*, in thier study, results revealed that ciprofloxacin induced silver nanoparticles possess better antimicrobial activities as compared to ciprofloxacin and silver nanoparticles alone.

Mohsen *et al*., (2020) combined ciprofloxacin with silver nanoparticles synthesized through a chemical method using; sodium borohydride and citrate, and a green synthesis approach using lactose. *S. aureus* and *E. coli* were tested for their sensitivity against the nanoparticles. The results revealed that ciprofloxacin-AgNPs have higher antibacterial activities as compared to pure AgNPs, with lactose-mediated AgNPs showing enhanced antibacterial activities. However, ciprofloxacin-AgNPs (citrate) showed uppermost inhibition zones against both strains than lactose and sodium borohydride ciprofloxacin AgNPs.

Ciprofloxacin (positive control) presented larger inhibition zone than pure AgNPs but smaller than those presented by the association of ciprofloxacin and AgNPs.

Wan *et al*., (2016) evaluated combinatorial effects of rifampicin, tigecycline and polymyxin B with AgNPs against multidrug resistant *Acinetobacter baumannii* using mice as test organisms. The results showed that all combination treatments have antibacterial activities with minimum inhibitory concentration of 0.25 µg/mL for polymyxin B association, 3.12 µg/mL for rifampicin and tigecycline associated silver nanoparticles. Moreover, it was found that in vivo, antibiotic-AgNPs combination establish better survival rates in *A. baumannii*-infected mice than single drug treatment. Ipe *et al*., (2020) investigated the effectiveness of combining several antibiotics with AgNPs against several bacterial strains. It was documented that certain bacteria were resistant to AgNPs alone and antibiotics alone, however, when treated with combinations of AgNPs and antibiotics, they became susceptible. For instance, *S. aureus* was resistant to ampicillin, cefpodoxime and cefuroxime antibiotics alone and AgNPs alone. After the combination of 1 µg/mL of AgNPs with 10 µg/mL of antibiotics, the effectiveness of antibacterial activities increased synergistically from no inhibition to susceptible range.

Lateef *et al*., (2016a) evaluated the use of cobweb of spiders for synthesis of AgNPs, and evaluated the synthesized nanoparticles for their antimicrobial efficacy against multidrug resistant bacterial isolates (*E. coli, K. granulomatis, P. aeruginosa, S. aureus*), as well as their potential application as additives in paints. Although the bacterial isolates showed some resistance to several antibiotics, at concentrations of 80 and 100 µg/mL, AgNPs effectively inhibited the growth of *E. coli, S. aureus and K. granulomatis*. In addition, the inclusion of 100 µg/mL of AgNPs improved the antibacterial activities of antibiotics (with percentage

improvement in the range of; ofloxacin: 3.1-20%, cefixime: 3.9-100% and augmentin: 6.3-100%) at concentrations of 1 mg/mL and 500 µg/mL respectively. Moreover, the application of biosynthesized AgNPs as additives in paint may improve the quality of the paint as it was shown that at 5 µg/mL, AgNPs have antibacterial and antifungal activities against *E. coli, P. aeruginosa*, and *Aspergillus niger, Aspergillus flavus* respectively.

Lateef *et al*., (2016b) evaluated antioxidant activities, mosquito larvicidal activities and antimicrobial potential of synthesized cocoa pod husk extract mediated silver nanoparticles (CPHE-AgNPs) against multidrug resistant clinical bacteria. At concentration of 40-100 µg/mL, CPHE-AgNPs effectively inhibited the growth of multidrug resistant isolate of *K. pneumonia* and *E. coli* with zones of inhibition of 10-14 mm. Several isolates were not inhibited by antibiotics, however, the combination of AgNPs with antibiotics produced inhibition effects against *K. pneumonia* and *E. coli*. In addition, CPHE-AgNPs contributed 42.9-100% improvement in antibacterial activities of ampicillin and cefuroxime.

In similar cases, Azeez *et al*., (2017) investigated antimicrobial, larvicidal and blood anticoagulant activities of cocoa bean extract mediated silver nanoparticles (CBE-AgNPs). With zones of inhibition of 10-14mm, multidrug resistant strains of *K. pneumoniae, S. aureus* and *E. coli* were effectively inhibited by CBE-AgNPs at concentrations of 40–100 µg/mL. Also, the nanoparticles were observed to improve the antimicrobial potency of antibiotics (erythromycin, cefuroxime, ampicillin and cefixime) by 42.9-100%.

2.5 Biomedical application of nanoparticles

According to Lateef *et al*., (2019), silver nanoparticles have been proven to be antiviral agents against several viruses such as *Herpesviridae, Arenaviridae, Retroviridae, Poxviridae*

Paramyxoviridae, Orthomyxoviridae and *Hepadnaviridae*. Access of viruses into host cells are blocked by nanoparticles having multivalent connections with components of viral surface and cell membrane receptors. As antiviral agents, they act directly on viral particles, bind with viral protein coat and disrupt their structure and function (Lateef *et al*., 2019). Orlowski *et al*., (2018) showed the effectiveness of tannic acid mediated AgNPs (TA-AgNPs) against herpes simplex virus 2 in a mouse. The virus was introduced initially into the mouse through the vagina to determine the immunity after treatment of initial infection with the nanoparticles. After the re-challenge with the virus, the infected mouse showed improved clinical sores and lower viral titre in vaginal tissue.

Metallic nanoparticles have thrombolytic activities, Dhandapani and Iyer, (2018) investigated thrombolytic activities of *Cassia auriculata*-AgNPs on human blood. Within 45 minutes of incubation, the nanoparticles dissolved the blood clots. Roy *et al*., (2019) investigated thrombolytic activities of *Euphorbia acruensis latex*-AgNPs on human blood clots, the nanoparticles dissolved blood clots within 30 minutes with suggestions that nanoparticles may inhibit enzymes that generate blood clotting proteins, and that nanoparticles prevent the conversion of prothrombin into thrombin, a key factor for producing insoluble strands of fibrin. In addition, the similar study suggested that nanoparticles may play a role in activating enzymes that produce plasmin which can break cross-links of fibrin molecules and dissolve blood clots.

Previous studies have proven nanoparticles as potential anticancer agents. For instance, Saratale *et al*., (2018) synthesized AgNPs using *Taraxucum officinale* extracts, the nanoparticles were found to have elevated cytotoxic activities against human liver cancer cells (HepG2). AgNPs and AuNPs synthesized using *Commelina nudiflora* extracts reduced

the viability of cells and improved cytotoxicity HCT-116 colon cancer cells (Kuppusamy *et al*., 2016). Copper oxide (CuONPs) nanoparticles were synthesized using a chemical process, and leaves extracts of *M. oleifera, Murraya koenigii, Tamarindus indica, Azadirachta indica and Hibiscus rosa-sinensis.* CuONPs were found to have synergy against four cancer cell lines; human breast, cervical, lung, epithelioma and one normal human dermal fibroblast cell line. In comparison, green synthesized nanoparticles exhibited better cytotoxic activities than chemically synthesized (Rehana *et al*., 2017).

In addition to improve the quality of paint, nanoparticles have been documented to be useful in controlling malaria by killing the larvae of the vector parasite. Silver nanoparticles synthesized using cocoa pod husk extracts (10-100 µg/mL) showed potent larvicidal activities of 70-100% against *Anopheles* mosquito within 2 hours, with LC_{50} of 43.52 µg/mL. Moreover, it was shown that the presence of the nanoparticles in paint leads to growth inhibition of *E. coli, K. pneumonia, S aureus, S, pyogenes, P. aeruginosa, A. flavus, A. fumigatus* and *A. niger* (Lateef *et al*., 2016b). Azeez *et al*., (2017) reported that against *Anopheles* mosquito larvae, silver nanoparticles of cocoa bean extracts had larvicidal activities within 3 hours at concentration of 10-100 µg/mL, displaying LC_{50} of 44.37 µg/mL. Roopan *et al*., (2013) evaluated larvicidal activity of *Cocos nucifera* extracts mediated silver nanoparticles against *Anopheles stephensi* and *Culex quinquefasciatus,* displaying an LC_{50} of 87.24 mg/L and 49.89 mg/L respectively, nanoparticles showed larvicidal activities.

Equally important, the inclusion of CBE-AgNPs into paint inhibited the growth of both bacterial and fungal isolates used (bacterial isolates: *E. coli, K. pneumoniae, S. pyogenes, S. aureus, P. aeruginosa* and fungal isolates: *A. flavus, A. fumigatus, A. niger*). In addition, it was shown that CBE-AgNPs have anticoagulating activities since they prevented the

coagulation of human blood in vitro and retained the morphology of the normal red blood cells after treatment (Azeez *et al*., 2017). Moreover, LC_{50} of 42.19 µg/mL was reported for *Bacillus safensis* extract mediated silver nanoparticles with 100% mortality of anopheline larvae within 12 hours (Lateef *et al*., 2015).

Silver nanoparticles possess anticoagulant activities. Leaf extract mediated silver nanoparticles have been tested for anticoagulation activities by several literature, and it has been documented that nanoparticles prevent coagulation of human blood. Upon microscopic examination of treated blood samples, red blood cells have been seen to retain undestroyed morphology (Lateef *et al*., 2018; Aina *et al.*, 2019; Roy *et al*., 2019). Furthermore, AgNPs have been documented to have antidiabetic activities. For instance, Prabhu *et al*., (2018) evaluated antidiabetic activities of AgNPs synthesized using leaves extracts of *Pouteria sapota* in streptozotocin-induced diabetic rats. Nanoparticles exhibited significant antidiabetic activities as there was an efficient reduction in blood sugar levels in rats after treatment.

2.6 Use of animal metabolites and agro-wastes for synthesis of metal nanoparticles

Biosynthesis of nanoparticles also involves use of animal metabolites. For example, Lateef *et al*., (2016a) synthesized AgNPs using spider cobweb extracts. The biosynthesized nanoparticles were found to possess antimicrobial activities against multidrug resistant bacterial strains. In addition, they were found to potentiate the activities of cefixime, augmentin, and ofloxacin. The cobweb mediated AgNPs were further reported to be useful in improving the quality of the paint as they inhibited the growth of fungi and bacteria. By adding silver nitrate and ammonia solution to boiled honey bee, Singh *et al*., (2014)

synthesized AgNPs which were found to have antimicrobial activities against methicillin resistant *S. aureus.* In another study, Sreelakshmi *et al*., (2011) reported biosynthesis of gold nanoparticles (AuNPs) by reacting honey solution with gold chloride. Biosynthesized AuNPs showed good inhibitory activities with MIC value of 31.25 µg/mL against *S. aureus* (MTCC 1144), for *C. albicans* (MTCC 183), *S. mutans* (MTCC 890), *E. coli* (MTCC 739) and *S. typhi* (MTCC 733), the MIC value obtained was 62.5 µg/mL.

Velmurugan *et al*., (2014) synthesized silver and gold nanoparticles using cashew nut shell liquid extracts, the nanoparticles were found to have antibacterial activities against fish pathogens (*Pseudomonas fluorescens-MTCC 67, Aeromonas hydrophila-MTCC 646, Edwardsiella tarda-MTCC 2400* and *Aeromonas bestiarum-MTCC 10106*). Nisha *et al.,* (2014) reported on lemon peel extracts mediated AgNPs that displayed good anti-dermatophytic activity against multidrug resistant *C. albicans* and *Trichophyton mentagrophytes*. Banana peel extracts mediated AgNPs synthesized by Ibrahim (2015) inhibited the growth of *E. coli, S. aureus, P. aeruginosa* and *B. subtilis.* Also, they improved antibacterial activities of levofloxacin against test organisms.

Patra and Baek (2015) used watermelon rind extracts to synthesize AuNPs that exhibited potential antibacterial activities against food borne pathogens; *B. cereus* ATCC 13061, *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 49444, *E. coli* ATCC 43890 and *S. typhimurium* ATCC 43174. In addition, the combination of AuNPs with 5 µg/mL of rifampicin and kanamycin separately let to improvement in antibacterial activities. The particles also showed potential antioxidant activities and anti-proteasome inhibitory activities, showing potential anticancer agent. Velu *et al*., (2015) synthesized AgNPs using groundnut (*Arachis hypogaea*) peel aqueous extracts. The nanoparticles showed good

larvicidal activity against dengue (*Aedes Aegypti*) and malaria (*Anopheles stephensi*) vectors, displaying LC_{50} of 1.85 and 3.13 ppm respectively.

Akintayo *et al*., (2020) synthesized silver nanoparticles using animal fur (goat) extracts and investigated their biological activities. It was found that the nanoparticles have anticoagulant, thrombolytic and larvicidal activities. Animal fur mediated silver nanoparticles (AF-AgNPs) prevented formation of blood clots and returned normal disc-shaped red blood cells. AF-AgNPs showed thrombolytic activities by 25% of lysis of preformed blood clots after 90 minutes. With concentration of 60-100 µg/mL, AF-AgNPs showed excellent larvicidal activities against *Anopheles* mosquito's larvae, displaying 100% mortality after 12 hours. Since the nanoparticles inhibited cell growth at high concentration, it was deduced that they can be explored in anticancer drugs with caution due to AF-AgNPs potential to induce chromosomal abberations.

3. Research methods

3.1 Research design

The study adopted a mixed method i.e., both qualitative and quantitative methods. Qualitative method was employed for identification of plant materials and silver nanoparticles that were more effective against *E. coli* and *S. aureus* whereas quantitative method was employed for analysis of data collected from antibacterial activities (inhibition zones).

3.2 Procedures

3.2.1 Preparation of *M. oleifera* leaf, seed and root extracts

Plant materials of *M. oleifera* were collected from Cimbebasia, Windhoek, Namibia. Plant extracts were prepared according to Mofolo *et al*., (2020) with some modifications. *Moringa* leaves, seeds and roots were collected, washed with double distilled water to remove dust particles and were then placed in a dark room for 2 weeks to let them dry. After drying, the samples were crushed into powder using a mortar, pestle and blender, then 20 g each was added in 100 mL of distilled water, methanol and ethanol respectively. The mixtures were then shaken on an orbital shaker for 72 hours. The filtrates were obtained using Whatman No.1 filter paper and then the extracts were concentrated using rotary evaporator (image shown in appendices). The dried extracts were weighed and refrigerated at 4 ºC for further studies.

3.2.2 Synthesis of silver nanoparticles

A modified method of Das *et al*., (2017) was used to synthesize silver nanoparticles. 100 mL of 5 mM silver nitrate ($AgNO_3$) solution was prepared. Then, 100 mg of dried plant extracts was added to $AgNO_3$ solution at room temperature (28.0 ± 2.0 °C). After the addition, the mixture was left on a magnetic stirrer at 150 rpm over night at 30 °C to allow bio-reduction process. In addition, silver nanoparticles were prepared in combination with each antibiotic (2.6 mg/mL), penicillin G and streptomycin separately. The ratio of silver nanoparticles to antibiotic was optimized at 1:0.5 on a volume to volume basis. This means that 10 mL of AgNPs-extract solution was added to 5 mL of antibiotic solution.

3.2.3 Characterization of silver nanoparticles

3.2.3.1 UV-vis Spectroscopy

The protocol of Adebayo-Tayo *et al*., (2019) was adopted to measure the optical absorbance of biosynthesized silver nanoparticles. UV-vis spectrophotometer analysis was done at room temperature using UV-1600PC spectrophotometer (image in appendices). The absorbance of the samples was read at wavelength of 200-800 nm and distilled water was used as a blank.

3.2.3.2 Fourier transform infrared spectroscopy

This method was done with reference to Kambale *et al*., (2020). The sample powder was directly spotted on the eye-piece of PerkinElmer FTIR spectrophotometer, then the analysis was carried out using the scanning range of 4000-450 cm^{-1} and resolution of 4 cm^{-1}. Attenuated total reflection method was used for analysis.

3.2.3.3 Scanning electron microscopy

Scanning electron microscopy (SEM) was done according to Das *et al*., (2017). Thin films of the sample were prepared by placing a small amount of the sample on the grid and was left to dry up. After, the sample was gold coated using Quorum Q 150R ES Carbon Coater and the images were taken.

3.2.4 Test Organisms

Bacterial strains used in this study were gram negative *E. coli* and gram positive *S. aureus* purchased from American Type Culture Collection company. Standard bacterial strains of *E. coli* (ATCC 33849) and *S. aureus* (ATCC 25923) were tested for antibacterial activities of plant extracts. Antibiotic resistant strains of *E. coli* (ATCC 35218) and *S. aureus* (ATCC 12489) were used to test AgNPs antibacterial activities. *E. coli* (ATCC 35218) is a β-

lactamase producer. The strain contains *bla*$_{\text{CMY-2}}$ genes that encodes an *ampC*-type β-lactamase that hydrolyses antibiotics, rendering them ineffective. The strain also contains *tet* genes that encode efflux proteins that extrude antimicrobial agents out of the cell, resulting in low intracellular concentration not sufficient enough to elicit an effect. Additionally, the strain contains *str*A, *str*B and *aac*A genes that encode phosphotransferases and acetyltransferases, enzymes that modify antibiotics, preventing them from binding to their targets.

S. aureus (ATCC 12489) contains an antibiotic resistant gene called *mecA* that encodes penicillin-binding protein (PBP) known as PBP 2a which reduces affinity for PBP. PBP2a remains active in the presence of β-lactam antibiotics that inhibit PBP enzymes, thus substituting for their functions in cell wall synthesis and allowing growth in the presence of the β-lactam inhibitors. In addition, *aac* genes present encode acetyltransferases, enzymes that modify antibiotics and render them inactive. The *blaZ* gene encodes a β-lactamase enzyme that hydrolyses a β-lactam ring and render β-lactam antibiotics inactive.

3.2.5 Screening for antibacterial activities

The procedure was done following the method of Widdatallah *et al*., (2020) using disc diffusion method. Petri plates were prepared by pouring Mueller Hinton agar which was left to solidify, then an inoculum culture was spread on the media surface. After, 6 mm paper discs impregnated separately with plant extracts and silver nanoparticles were placed on agar surface. The plates were incubated at 37 °C overnight. Zones of inhibitions around the discs were measured using a ruler in millimeters. Bacterial cultures were prepared to turbidity equivalence of 0.5 McFarland standard using a spectrophotometer. Distilled water was used

as a negative control while streptomycin as a positive control. The tests were done in triplicates.

3.2.6 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations were determined using the protocol of Debalke *et al*., (2018) with some modification using a micro-dilution method by 96-well microtiter plate. For each plate, 100 µL of nutrient broth was placed in each well, followed by the addition of 100 µL of plant extract mediated silver nanoparticles in the first column of the microplate. Starting from column 1, serial dilution was conducted up to 10^{th} column with double folding and the final volume of 50 µL was discarded. Then, 50 µL of each bacterial strain was aseptically added to each well. The 11^{th} column saved as a positive control to which streptomycin was added while column 12 was the negative control containing distilled water. The plates were incubated at 37 °C for 24 hours. MIC was determined by the addition of 30 µL of 0.02% of tetrazolium blue chloride after 24 hours, then plates were further incubated for 1 hour at 37 °C. The wells that did not change colour after final incubation represented no growth of bacteria and was considered as the MIC.

3.2.7 Data analysis

The experimental data were expressed as mean ± standard error of the mean (SEM). Data collected were analysed using SPSS software version 26 where means of treated and control groups were compared. The data was first tested for normality using Shapiro-Wilk test in order to identify whether the data is normally distributed or not. Provided that it is normally distributed, one-way ANOVA was used to test the hypothesis. However, if the data fails

normality test, Kruskal Wallis test was to be used. A significant probability value of 0.05 was used ($p = 0.05$).

3.2.8 Research Ethics

A collection permit was obtained from Ministry of Environment and Tourism for permission of plant collection. Ethical clearance and permission to conduct the research was obtained from University of Namibia, Research Ethics Committees and Research and Publication offices respectively.

4. Results

4.1 Synthesis of silver nanoparticles

The formation of silver nanoparticles was observed visually by colour change. After the bio-reduction process, the colour of the solution changed from yellow to dark brownish-black, which is an indication of formation of AgNPs. The intensity of the colour was observed to increase with incubation time. In Fig. 4.1, A) $AgNO_3$ solution, B) mixture of $AgNO_3$ solution and dried plant extracts before incubation time and C) after incubation time, where the reduction of silver ions was vividly evident from change of colour. Since there was colour change after the addition of plant extracts, it was found that *M. oleifera* extracts reduce silver ions and therefore play an important role in formation of silver nanoparticles. Formation of silver nanoparticles is due to excitation of surface plasmon resonance in the reaction mixture.

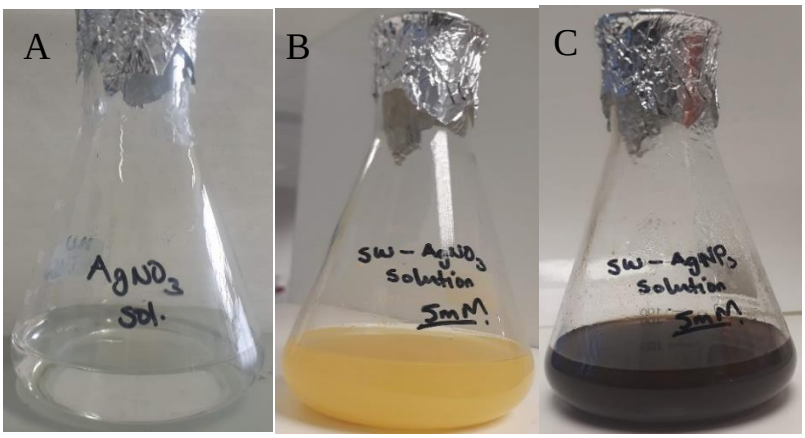

Figure 4.1. Change of colour indicates formation of AgNPs. A: $AgNO_3$ solution. B: $AgNO_3$ + *Moringa* plant extract at 0 hours. C: $AgNO_3$ + *Moringa* plant extract after 24 hours. Dark brown-black colour indicates the formation of AgNPs.

4.2 Characterization of silver nanoparticles

4.2.1 UV-vis spectroscopy analysis of as synthesized silver nanoparticles

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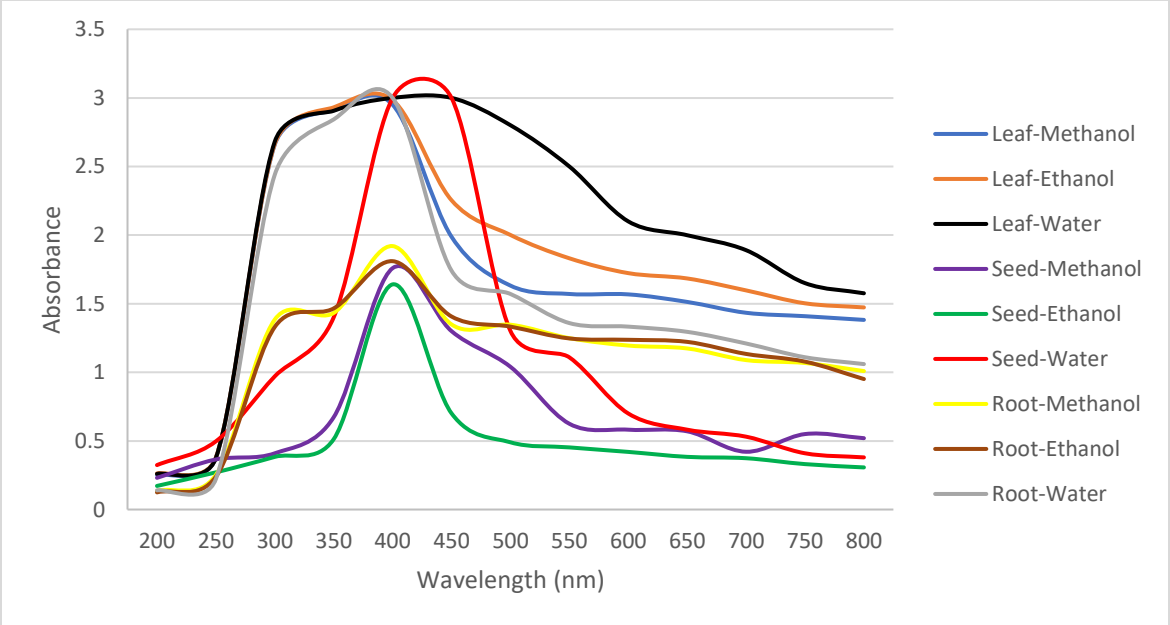


Figure 4.2. UV-vis spectrum of synthesized AgNPs.

Figure 4.2 shows UV-vis absorption spectra of synthesized AgNPs at the end of reaction time in the range of 200-800 nm wavelength. Maximum absorption peak appeared around 400-440 nm for all extracts mediated silver nanoparticles, indicating the formation of silver nanoparticles in this study as previous literature suggests that surface plasmon resonance maximum peak of 400-500 nm is a characteristic of AgNPs formation.

4.2.2 Fourier transform infrared spectroscopy

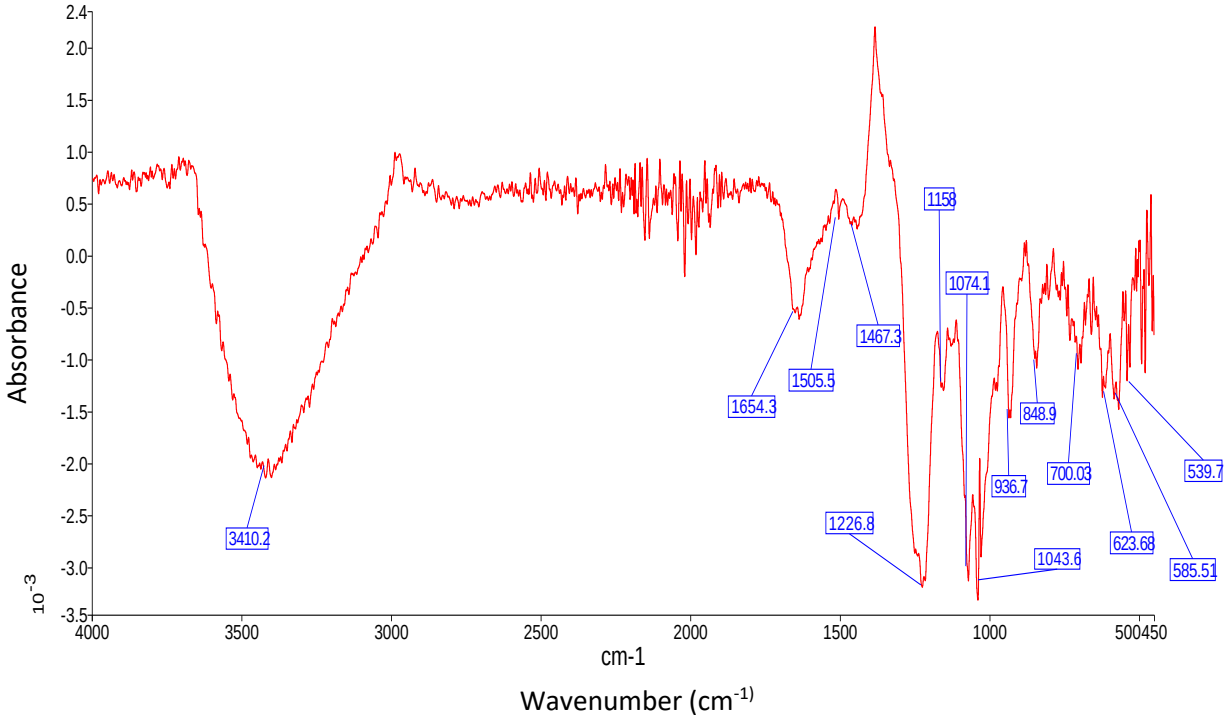


Figure 4.3. Fourier transform infrared spectroscopy analysis of leaves-AgNPs.

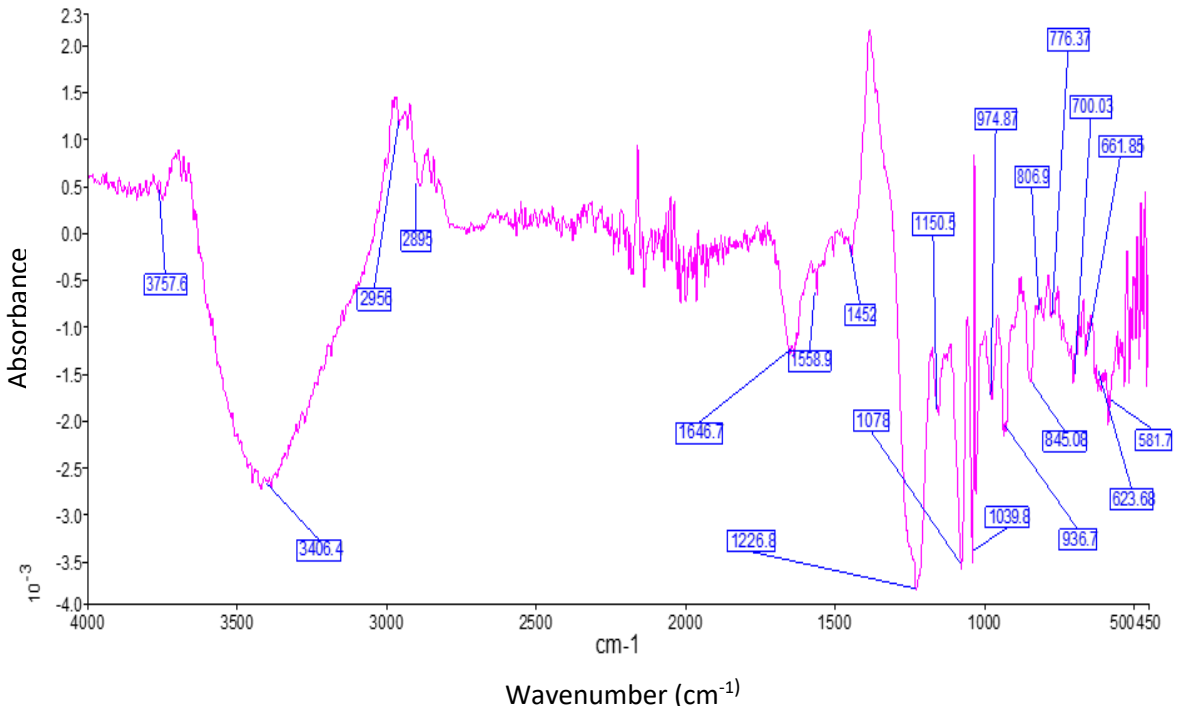


Figure 4.4. Fourier transform infrared spectroscopy of seeds-AgNPs.

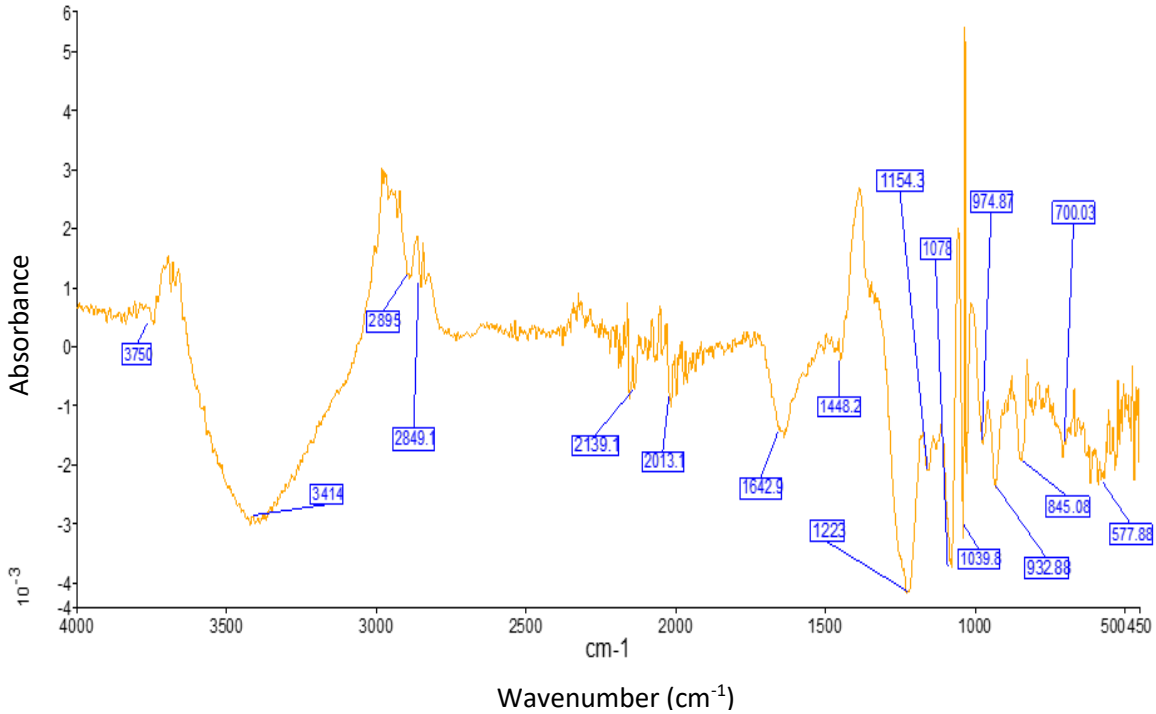
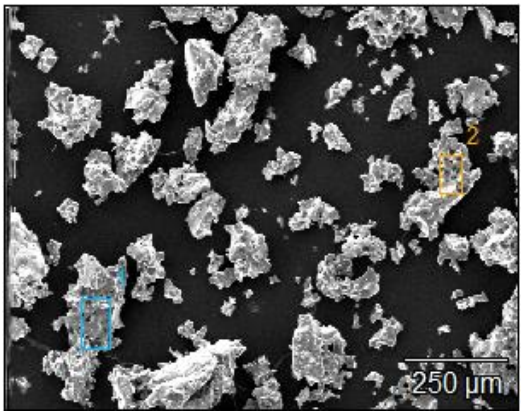


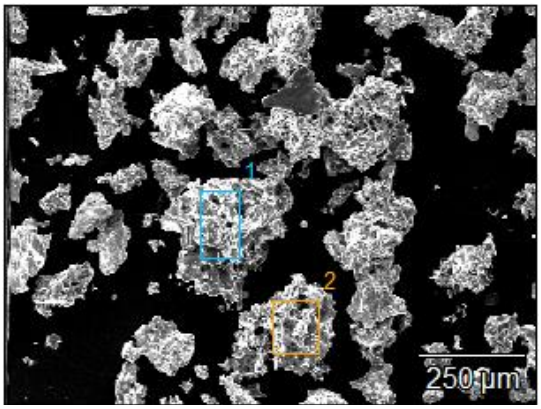
Figure 4.5. Fourier transform infrared spectroscopy analysis of roots-AgNPs.

Figure 4.3, 4.4 and 4.5 shows FTIR analysis of synthesized AgNPs of leaves, seeds and roots carried out to find out functional groups that might be responsible for binding and reducing silver ion (Ag^{+}). FTIR analysis revealed prominent peaks at 3410.2, 1654.3, 1226.8, 1074.1 and 1043.6 cm^{-1} for leaves-AgNPs. For seeds-AgNPs, prominent peaks were recorded at 3406.4, 1646.7, 1226.8, 1078.0 and 1039.8 cm^{-1}, whereas peaks at 3414.0, 1642.9, 1223.0, 1078.0 and 1039.8 cm^{-1} were obtained for roots-AgNPs. The peaks at 3410.2, 3406.4 and 3414.0 cm^{-1} are known to be associated with stretching vibrations of hydroxyl (OH) functional group in alcohols or phenolic compounds. The peaks at 1654.3, 1646.7 and 1642.9 cm^{-1} might be attributed to the presence of carbonyl (C=O) group of amides I and II from proteins. Peak 1226.8, 1226.8 and 1223.0 cm^{-1} show C-N bond in aliphatic amines. Peaks 1074.1, 1043.6, 1078.0,1039.8, 1078.0 and 1039 cm^{-1} indicate the presence of C-O stretch which may be due to ether linkage.

4.2.3 Scanning electron microscopy

The sizes, shapes and morphology of synthesized AgNPs were revealed by SEM in fig. 4.6. The results showed sizes of approximately 46.49 ± 0.73 nm, 54.53 ± 0.98 nm and 60.96 ±1.17 nm for leaves-AgNPs (A), seeds-AgNPs (B) and roots-AgNPs (C) respectively. Leaves-AgNPs were observed to be well dispersed as compared to seeds-AgNPs and roots-AgNPs where partial agglomeration was observed. SEM images showed AgNPs of different types of shapes without association. In addition, they are crystalline in nature. The energy dispersive x-ray (EDX) analysis (Fig. 4.7-4.9) showed the presence of silver in synthesized AgNPs predominantly in leaves mediated silver nanoparticles.

A) 

B) 

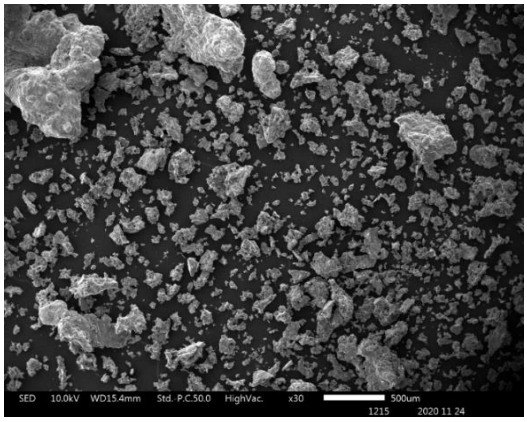
C) 

Figure 4.6. SEM images of *M. oleifera* leaves (A), seeds (B), and roots (C) extracts mediated AgNPs.

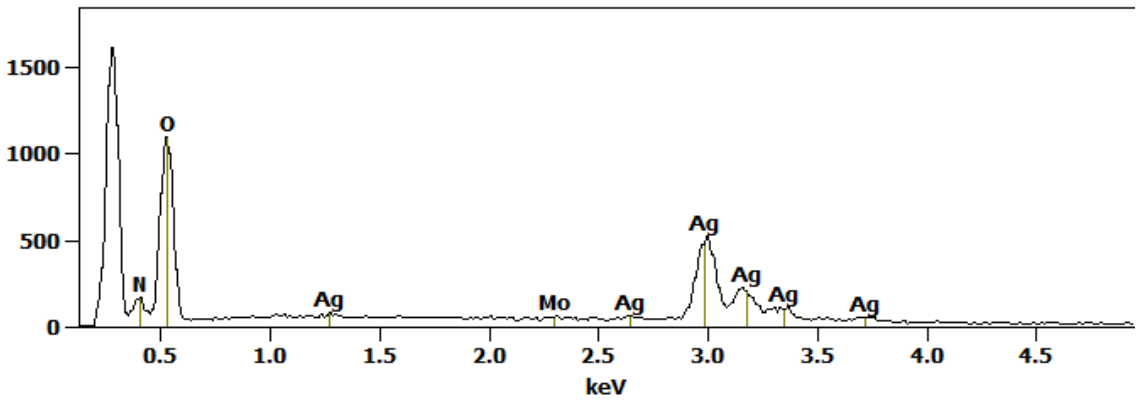


Figure 4.7. Energy dispersive x-ray (EDX) analysis of *M. oleifera* leaves extract mediated AgNPs.

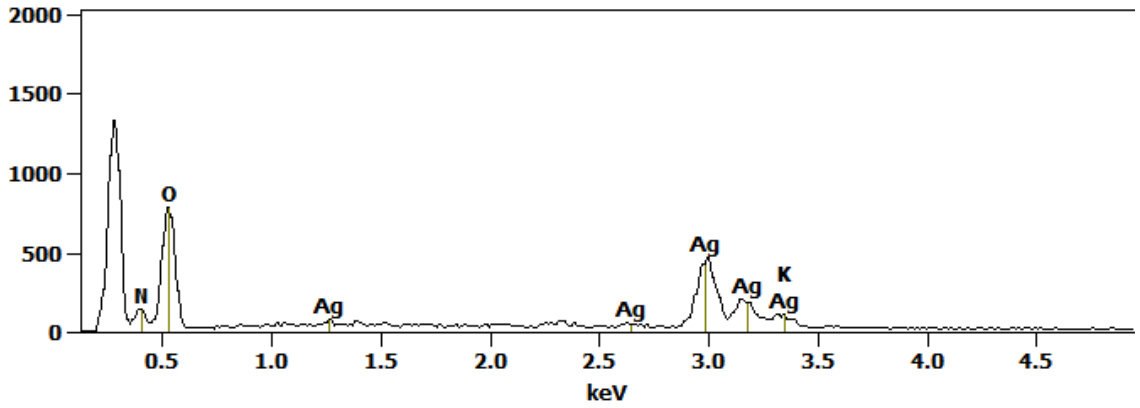


Figure 4.8. Energy dispersive x-ray (EDX) analysis of *M. oleifera* seeds extracts mediated AgNPs.

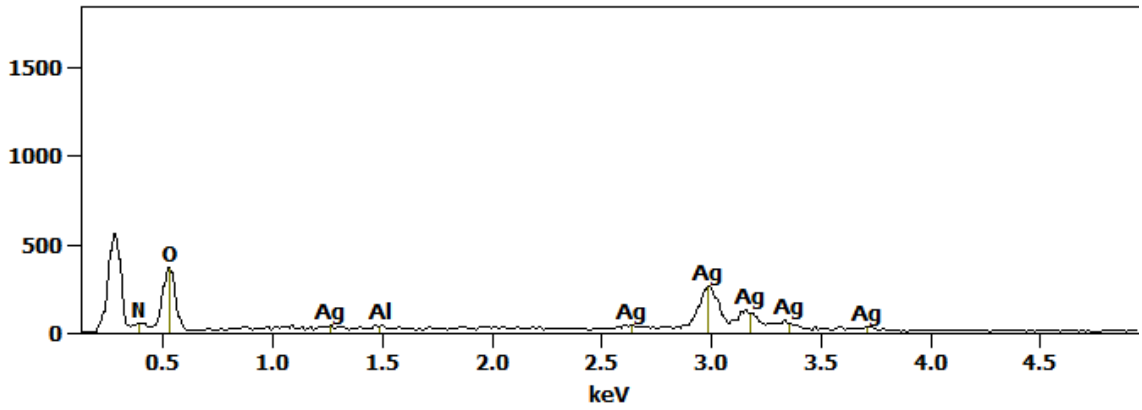


Figure 4.9. Energy dispersive x-ray (EDX) analysis of *M. oleifera* roots extract mediated AgNPs.

4.3 Antibacterial activities of *M. oleifera* leaf, seed and root extracts

In this study, standard strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 33849) were used to assess the antibacterial activities of leaves, seeds and roots extracts of *M. oleifera*. All plant extracts showed activities against test bacterial strains

(sample plates in appendices). Among all the extracts, the results revealed a concentration-dependent inhibition on both bacteria, with *S. aureus* being more sensitive than *E. coli*.

Table 4.1. Antibacterial activities of methanol, ethanol and aqueous extracts of *M. oleifera* leaves on test organisms.

Test Organisms	Zone of inhibition (mm) ± SE								
	LM (mg/mL)			LE (mg/mL)			LW (mg/mL)		
	200	150	100	200	150	100	200	150	100
S. aureus	11.3 ± 0.33	9.67 ± 0.33	8.33 ± 0.33	12.67 ± 0.33	11.33 ± 0.33	9.67 ± 0.33	9.33 ± 0.33	7.66 ± 0.33	6.67 ± 0.33
E. coli	10.33 ± 0.33	8.33 ± 0.88	7.33 ± 0.33	10.67 ± 0.33	8.00 ± 0.58	7.67 ± 0.33	7.50 ± 0.29	6.6 ± 0.33	0.00 ± 0.00

Key: LM = Leaf methanolic extract. LE = Leaf ethanolic extract.

LW = Leaf aqueous extract.

Leaves extracts showed antibacterial activities against both strains with ethanolic extracts being the most effective, and against *S. aureus*. The inhibition zones increase with increasing concentrations of extracts. The highest inhibition zone was observed at concentration of 200 mg/mL of ethanolic extracts against *S. aureus* (12.67 ± 0.33 mm) while the lowest was against *E. coli* where aqueous extracts did not show any activity at concentration of 100 mg/mL.

Table 4.2. Antibacterial activities of methanol, ethanol and aqueous extracts of *M. oleifera* seeds on test organisms.

Test Organisms	Zone of inhibition (mm) ± SE								
	SM (mg/mL)			SE (mg/mL)			SW (mg/mL)		
	200	150	100	200	150	100	200	150	100
S. aureus	11.33 ± 1.20	10.67 ± 0.88	9.66 ± 0.33	11.00 ± 0.58	10.33 ± 0.88	9.66 ± 0.33	9.33 ± 0.33	8.33 ± 0.67	7. 00 ± 0.58
E. coli	10.83 ± 0.44	8.00 ± 1.00	7.00 ± 1.00	8.66 ± 0.88	7.66 ± 0.88	0.00 ± 0.00	8.33 ± 0.88	7.66 ± 0.33	6.67 ± 0.33

Key: SM = Seed methanolic extracts. SE = Seed ethanolic extract.

SW = Seed aqueous extracts.

Seed extracts also revealed to be effective against all test bacteria with the highest activity possessed by the methanolic extracts against *S. aureus* (11.33 ± 1.20 mm), and the lowest with aqueous extracts against *E. coli* (6.67 ± 0.33 mm). These results also showed a concentration-dependent inhibition zones in both bacteria.

Table 4.3. Antibacterial activities of methanol, ethanol and aqueous extracts of *M. oleifera* roots on test organisms.

Test Organisms	Zone of inhibition (mm) ± SE		
	RM (mg/mL)	RE (mg/mL)	RW (mg/mL)

	200	150	100	200	150	100	200	150	100
S. aureus	8.67 ± 0.88	8.33 ± 0.33	0.00 ± 0.00	8.17 ± 0.17	7.07 ± 0.07	0.00 ± 0.00	9.67 ± 0.33	7.33 ± 0.33	7.00 ± 1.00
E. coli	8.67 ± 0.88	8.30 ± 0.33	6.66 ± 0.33	7.33 ± 0.33	6.67 ± 0.33	0.00 ± 0.00	9.39 ± 0.57	7.33 ± 0.37	6.67 ± 0.33

Key: RM = Root methanolic extracts. RE = Root ethanolic extracts.

RW = Root aqueous extracts.

Root extracts possess antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. The highest zone of inhibition was acquired against gram positive *S. aureus* (9.67 ± 0.33 mm) while the lowest was exhibited against both *S. aureus* and *E. coli* (0.00 ± 0.00 mm).

Among plant materials, leaves showed to be the most effective followed by seeds and lastly roots based on their highest inhibitory activities of 12.67 ± 0.33 mm, 11.33 ± 0.33 and 9.67 ± 0.33 mm respectively, all of which were recorded at concentration of 200 mg/mL. All inhibition zones exhibited were smaller than those of the positive control streptomycin which presented inhibition zones that ranged between 19.00 ± 0.33 mm and 21.67 ± 0.45 mm.

Table 4.4. Test for normality of inhibition zones data set for *M. oleifera* plant extracts.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Triplicates	.214	54	.000	.849	54	.000

After testing the data for normality using SPSS, the data were found to deviate from normal distribution, hence the significant value of 0.000 which is less than the probability value of 0.05.

Table 4.5. Kruskal Wallis test for inhibition zones data set for *M. oleifera* plant extracts.

Test Statistics[a,b]

	Triplicates
Kruskal-Wallis H	9.232
df	1
Asymp. Sig.	.002

After analyzing the data using Kruskal Wallis, the significant value of 0.002 was obtained. This means that the null hypothesis is rejected while the alternative hypothesis is accepted.

4.4 Antibacterial activities of silver nanoparticles

Synthesized silver nanoparticles were evaluated for their antibacterial activities against multi drug resistant strains of *S. aureus* (ATCC 12498) and *E. coli* (ATCC 35218). In addition, the synergistic effects of the combination of AgNPs with antibiotics were also assessed against test bacterial strains. In this study, a concentration of 5 mM of AgNPs and 2.6 mg/mL of each antibiotic (penicillin G and streptomycin) was used. AgNPs synthesized using all plant material; leaves, seeds and roots showed inhibition activities against both gram positive and gram negative bacteria (petri plates in appendices). The results are shown in table 4.6-4.14. The effectiveness of *M. oleifera* extract mediated AgNPs, penicillin G + *M. oleifera* extract mediated AgNPs, streptomycin + *M. oleifera* extract mediated AgNPs and streptomycin alone (positive control) were evaluated separately against test bacteria and it was found that in all plant materials, *M. oleifera* mediated AgNPs are effective against all

bacterial strains. However, their inhibition zones are smaller than those revealed by streptomycin alone. On the other hand, the results showed that the combination of antibiotics and plant extract mediated AgNPs have larger inhibition zones than those displayed by streptomycin alone (positive control).

Table 4.6. Antibacterial activities of *M. oleifera* leaf extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	18.67 ± 0.35	19.33 ± 0.33	16.66 ± 0.33	20.33 ± 0.88
E. coli	17.66 ± 0.33	18.33 ± 0.69	15.67 ± 1.20	19.67 ± 0.54

Among all solvents, *S. aureus* was more sensitive than *E. coli*. Leaf extracts mediated AgNPs had maximum inhibition zone of 19.33 ± 0.33 mm against *S. aureus* which was displayed by ethanol extracts. The lowest inhibition zone of 15.67 ± 1.20 mm was demonstrated by aqueous extracts with *E. coli*. All the inhibition zones shown by extract mediated AgNPs were smaller than those displayed by streptomycin.

Table 4.7. Antibacterial activities of *M. oleifera* seed extracts mediated AgNPs.

Test organisms	Zones of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	18.33 ± 0.33	17.00 ± 0.57	16.00 ± 0.57	19.60 ± 0.33
E. coli	15.33 ± 0.88	15.66 ± 0.67	16.67 ± 0.33	19.33 ± 0.32

Methanol extracts showed maximum inhibition zone of 18.33 ± 0.33 mm against gram positive *S. aureus* while the lowest zone of 15.33 ± 0.88 mm was obtained in *E. coli* with methanol solvent. In organic solvents, *S. aureus* was more sensitive than *E. coli*, however, aqueous extracts were more effective against gram negative *E. coli* than gram positive *S. aureus*.

Table 4.8. Antibacterial activities of *M. oleifera* root extract mediated AgNPs.

Test organisms	Zones of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	17.00 ± 1.53	16.67 ± 0.66	16.35 ± 1.45	19.68 ± 0.35
E. coli	14.33 ± 0.33	15.33 ± 0.66	15.67 ± 0.67	19.68 ± 0.67

Highest zone of inhibition (17.00 ± 1.53 mm) was displayed by methanol solvent for *S. aureus* while the lowest inhibition zone observed to be 14.33 ± 0.33 mm for *E. coli*.

Table 4.9. Antibacterial activities of penicillin G + leaf mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	21.83 ± 1.74	23.33 ± 0.88	21.33 ± 0.66	20.33 ± 0.33
E. coli	22.00 ± 1.00	22.30 ± 0.65	21.67 ± 0.88	19.00 ± 1.00

The combination of penicillin G and plant extracts produced larger inhibition zones than streptomycin alone. Only with ethanol solvent was gram positive more sensitive than gram negative. With methanol and aqueous solvents, the growth of *E. coli* was more inhibited compared to *S. aureus*. Both maximum (23.33 ± 0.88 mm) and minimum (21.33 ± 0.66 mm) zone of inhibition were exhibited against *S. aureus* by ethanol and aqueous extracts respectively.

Table 4.10. Antibacterial activities of penicillin G + seed extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	21.67 ± 0.88	22.33 ± 0.67	21.33 ± 0.66	20.00 ± 1.00
E. coli	21.33 ± 1.45	21. 67 ± 0.88	21.61 ± 2.19	19.60 ± 0.88

Highest zone of inhibition (ZOI) was displayed by ethanol extract (22.33 ± 0.67 mm) against *S. aureus* while a lowest ZOI (21.33 mm) was identical in both strains and exhibited by methanol in *E. coli* and aqueous extracts in *S. aureus*.

Table 4.11. Antibacterial activities of penicillin G + root extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	21.67 ± 0.88	21.33 ± 1.20	21.33 ± 0.67	20.35 ± 0.33
E. coli	21.33 ± 2.33	20.10 ± 0.09	20.60 ± 0.59	19.33 ± 0.33

S. aureus was most sensitive in all solvents with maximum ZOI of 21.67 ± 0.88 mm with methanol. Lowest ZOI of 20.60 ± 0.59 mm was acquired against *E. coli* with aqueous extracts.

Table 4.12. Antibacterial activities of streptomycin + leaf extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	25.33 ± 0.33	26.17 ± 1.88	24.67 ± 0.33	20.67 ± 0.35
E. coli	23.67 ± 0.33	25.00 ± 0.58	24.00 ± 0.00	19.33 ± 0.33

In each solvent, *S. aureus* was better inhibited than *E. coli*. Maximum inhibition zone was 26.17 ± 0.88 mm against *S. aureus* while lowest inhibition zone was 23.67 ± 0.33 mm against *E. coli*.

Table 4.13. Antibacterial activities of streptomycin + seed extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	25.33 ± 0.33	24.67 ± 0.88	25.00 ± 0.58	20.33 ± 0.67
E. coli	24.83 ± 0.44	22.35 ± 0.88	23.66 ± 0.88	19.67 ± 0.33

Methanol extract was most effective with zone of 25.33 ± 0.33 mm against the gram positive strain. Ethanol extract exhibited the least activity of 22.35 ± 0.88 mm against *E. coli.*

Table 4.14. Antibacterial activities of streptomycin + root extracts mediated AgNPs.

Test organisms	Zone of inhibition (mm)			
	Methanol	Ethanol	Aqueous	Streptomycin
S. aureus	21.67 ± 0.88	23.67 ± 0.67	22.67 ± 0.33	20.33 ± 0.67
E. coli	21.33 ± 0.67	22.67 ± 0.88	21.67 ± 0.88	19.00 ± 1.16

Aqueous extracts were most effective with zone of 24.67 ± 0.67 mm against gram positive *S. aureus,* methanol extracts exhibited the least activity of 21.33 ± 0.67 mm against gram negative *E. coli*.

Table 4.15. Test for normality for all antibacterial activities data set of AgNPs.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Inhibitions	.132	162	.000	.960	162	.000

After testing the data for normal distribution, it was found that the data was not normally distributed, hence the sig. value of 0.00 which is smaller than the probability value of 0.05.

Table 4.16. Kruskal Wallis test for all antibacterial activities data set of AgNPs.

Test Statistics[a,b]

	Inhibitions
Kruskal-Wallis H	120.027
df	1
Asymp. Sig.	.024

After analyzing data with Kruskal Wallis test, a significant value of 0.024 (less than probability value of 0.05) was obtained, this means that null hypothesis is rejected while alternative hypothesis is accepted.

4.5 Minimum inhibitory concentration of *M. oleifera* plant extracts.

Table 4.17. Minimum inhibitory concentration (MIC) of leaf extracts of *M. oleifera.*

Test organisms	MIC value (mg/mL)		
	Methanol	Ethanol	Aqueous

S. aureus	50.00 ± 0.45	50.00 ± 0.33	100.00 ± 0.55
E. coli	50.00 ± 1.25	50.00 ± 1.10	100.00 ± 0.45

All leaf extracts exhibited equivalent MIC value on both test bacteria, with methanol and ethanol showing lower MIC than aqueous extract.

Table 4.18. Minimum inhibitory concentration (MIC) of seed extracts of *M. oleifera.*

Test organisms	MIC value (mg/mL)		
	Methanol	Ethanol	Aqueous
S. aureus	50.00 ± 0.45	25.00 ± 0.50	100.00 ± 0.45
E. coli	50.00 ± 0.33	50.00 ± 0.50	N/A

Ethanol extract displayed lowest MIC value on *S. aureus* but equivalent to methanol on *E. col. E. coli* required a higher concentration of extract to be inhibited than *S. aureus* did.

Table 4.19. Minimum inhibitory concentration (MIC) of root extracts of *M. oleifera.*

Test organisms	MIC (mg/mL)		
	Methanol	Ethanol	Aqueous
S. aureus	50.00 ± 1.25	50.00 ± 0.45	N/A
E. coli	25.00 ± 1.10	50.00 ± 0.55	N/A

Aqueous extract of roots did not inhibit the growth of any of the test organisms, however, organic extracts were effective against the growth of both test organism. Methanol produced lowest MIC value on *E. coli* than *S. aureus.*

4.6 Minimum inhibitory concentration of silver nanoparticles.

Table 4.20. Minimum inhibitory concentration of *M. oleifera* mediated silver nanoparticles (Mo-AgNPs).

Test organisms	Leaf extracts		
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	50.0	25.0	50.0
E. coli	50.0	25.0	100.0
	Seed extracts		
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	25.0	25.0	25.0
E. coli	100.00	50.0	100.0
	Root extracts		
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	12.5	12.5	12.5
E. coli	50.0	25.0	50.0

Among all the plant material-based extracts, the lowest MIC value was obtained for *S. aureus* (12.5 mg/ml) with AgNPs of root extracts. In general, gram positive *S. aureus* was more susceptible to Mo-AgNPs than gram negative *E. coli*.

Table 4.21. Minimum inhibitory concentration of combination of *M. oleifera* mediated AgNPs + penicillin G (Mo-AgNPs-penicillin G).

Test organisms	Leaf extracts		
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	1.56	3.13	6.25
E. coli	12.5	12.5	12.5
Seed extracts			
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	12.5	12.5	25.0
E. coli	25.0	12.5	25.0
Root extracts			
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	6.25	6.25	12.5
E. coli	12.5	25.0	12.5

The combination of penicillin and Mo-AgNPs showed a lowest MIC value of 1.56 mg/mL, obtained against *S. aureus* with leaf extracts. For *E. coli*, lowest MIC value was 12.5 mg/mL, which was obtained from seed and root extracts. *S. aureus* was more sensitive to the treatment than *E. coli* was.

Table 4.22. Minimum inhibitory concentration of combination of *M. oleifera* mediated AgNPs + streptomycin (Mo-AgNPs-streptomycin).

Test organisms	Leaf extracts		
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	3.13	6.25	6.25
E. coli	12.5	12.5	25.0
Seed extracts			
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	12.5	6.25	12.5
E. coli	25.0	25.0	12.5
Root extracts			
	Methanol	Ethanol	Aqueous
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
S. aureus	6.25	6.25	6.25
E. coli	12.5	12.5	25.0

The lowest MIC value exhibited by the combination of streptomycin and Mo-AgNPs was 3.13 mg/mL, which was obtained for *S. aureus*. The lowest MIC for *E. coli* was 12.5 mg/mL, this shows that *S. aureus* was more sensitive to the combination than *E. coli*.

5 Discussion

Figure 4.1 shows successful synthesis of silver nanoparticles (AgNPs) using extracts of *M. oleifera* which was confirmed by colour change. After 24 hours of stirring on a magnetic

stirrer, the colour change from pale yellow to brownish-black suggested the conversion of silver ions into metallic silver that assembles into particles called AgNPs. This suggests that *M. oleifera* has potential bioactive compounds such as alkaloids and saponins that can reduce ionic silver in order to form AgNPs. The results are in line with the previous study of Oliveira *et al*., (2019) which stated that silver ions are reduced in the presence of plant extracts containing phytochemical constituents such as terpenoids, alkaloids and polyphenols. The time-dependent colour change is due to excitation of surface plasmon resonance (SPR) which promotes modification in the colour of suspension during the reaction process (Ahmed *et al*., 2015).

Krisna *et al*., (2020) defined surface plasmon resonance as the vibrations of electrons on the surface of nanostructures of metals created in response to external stimulus such as light. Several researchers have reported variation in the colour of synthesized AgNPs colloidal solution, such as pale yellow to dark brown and brown-black due to the composition of biomolecules responsible for synthesis of nanoparticles. The excitation of the reduced particles is influenced by the nature of the bio-reductant molecules present, which leads to the development of different colours (Lateef *et al*. 2016a; Lateef *et al*. 2016d).

Figure 4.2 represents surface plasmon resonance absorbance measured by UV-vis spectroscopy in order to confirm the formation of AgNPs. In the current study, the maximum absorption peaks of AgNPs were observed at different wavelengths in the range of 400-440 nm. This might suggest that prepared AgNPs interacted with different phytochemical constituents of varying concentration present in different plant parts. This leads to differences in surface resonance absorption signatures. The maximum absorption peaks

observed in this study signifies the formation of AgNPs as it was suggested by previous studies that the maximum surface plasmon resonance that ranges between 400-500 nm confirms the synthesis of AgNPs (Singh *et al*., 2019).

The broadness of the spectra may be an indication of fair incidence of polydispersed particles (Lateef *et al*., 2016d). The spectrum range reported in the current study is in great correlation with previous findings from various researchers. For instance, the maximum absorption peaks of 436 nm, 428.5 nm, 418 nm and 440 nm were obtained for AgNPs synthesized using cobweb, cocoa pod husk, *Chasmanthera dependens* and *Synsepalum dulcificum* respectively (Lateef *et al*., 2016a; Lateef *et al*. 2016b; Aina *et al*. 2019; Lateef *et al*. 2016d). Leaves mediated AgNPs displayed higher absorbance as a function of intense colour formation, suggesting the presence of higher abundance of biomolecules (Lateef *et al*, 2016d). Synthesized AgNPs with maximum absorption peak between 410-440 nm have desired sizes (Adil *et al*., 2019). Hence, it is further suggested that prepared AgNPs in the current study have sizes sufficient enough to render antibacterial activities.

Fourier transform infrared spectroscopy analysis revealed that identical functional groups in leaves, seeds and roots samples might be responsible for binding, stabilizing and reducing silver ions into AgNPs, results are presented in figure 4.3, 4.4 and 4.5. The dominant peaks shown by FTIR results suggests the presence of functional groups of hydroxyl, carbonyl and ether linkages which may belong to groups of phytochemical compounds (Sathyavathi *et al*., 2010; Moodley *et al*., 2018; Kambale *et al*., 2020; Lateef *et al*., 2016d). This signifies that bioactive compounds such as alkaloids, flavonoids, polysaccharides, terpenoids, phenols,

saponin and proteins present in *M. oleifera* may be responsible for stabilizing, reducing or capping of silver ions (Amabye & Tadesse, 2016; Elongavan *et al*., 2014; Etejere *et al*. 2015; Das *et al*., 2020). These results correlate with the previous study of Prasannaraj & Venkatachalam (2017) which observed similar functional groups on the surface of AgNPs produced from leaf extracts of medicinal plants, and suggested that bioactive compounds present in leaf biomass are potential silver ion capping agents. However, it was reported by Etejere *et al*., (2015) that main active compounds of *Moringa* are saponins, flavonoids and alkaloids, whose concentrations are high compared to other phytochemical ingredients. It is then suggested that the three active ingredients must have had the most profound role in reducing silver ions into silver nanoparticles. Regardless of the solvent used for extraction, FTIR analysis revealed that leaves, seeds and roots of *M. oleifera* are rich in biomolecules that served as bio-reductants in synthesis of AgNPs.

Figure 4.6 shows SEM images of synthesized AgNPs using *M. oleifera* extracts. *M. oleifera*-AgNPs were in size range of 46.49 nm to 60.96 nm, which is in agreement with earlier researchers (Kambale *et al*., 2020; Garibo *et al*., 2020; Vanlalveni *et al*., 2021; Das *et al*., 2013; Sathyavathi *et al*., 2010). The nanoparticles were crystalline in nature; however, different types of shapes were observed for plant extract mediated AgNPs. This may be caused by different solvents used or the concentration of silver nitrate that was used to prepare AgNPs, it has been reported that shapes of nanoparticles can be affected by increasing silver nitrate concentration. This is in line with Das *et al*., (2020) in which it was suggested that agglomeration can also alter the shapes of nanoparticles. In addition, seeds-AgNPs and root-AgNPs appeared to be partially aggregated. Similar results were observed

by previous researchers (Lateef *et al*., 2016d; Mofolo *et al*., 2020; Erdogan *et al*., 2019). However, in these aggregates, nanoparticles were not in permanent contact with each other as some were seen to be individually isolated and not in association with others. This correlates with the previous findings from several researchers. For instance, Singh *et al.*, (2019) synthesized aggregated AgNPs using aqueous extracts of *Premna integrifolia*. Nevertheless, it was stated that nanoparticles were stabilized by extracts of *P. integrifolia* that served as a capping agent since the particles were not directly attached to each other.

EDX analysis showed the presence of silver in synthesized nanoparticles, with leaves-AgNPs showing dominant peaks than seeds-AgNPs and roots-AgNPs, the results are presented in figure 4.7, 4.8 and 4.9. Among all nanoparticles, a strong silver signal peak was observed at 2.9 keV which is similar to previous research studies (Jagtap and Bapat, 2013), and is close to 3 keV, the optical absorption peak of silver (Singh *et al*., 2018). In addition, EDX showed the presence of oxygen atoms in the nanoparticle samples. This suggests the existence of phytochemical compounds on the surface of synthesized AgNPs, which supports the fact that silver nanoparticles from 3 different plant parts did not have similar absorption peaks in UV-vis spectroscopy (Kambale *et al*., 2020). Since plant secondary metabolites act as both capping and reducing agents of nanoparticles, the differences in organic compounds of the 3 different plant parts could be attributed to the observed variation in UV-vis absorption and sizes of synthesized nanoparticles (Bagur *et al*., 2019; Kambale *et al*., 2020). It can therefore be inferred that *M. oleifera* is a genuine source of biomolecules in the eco-friendly synthesis of AgNPs. There were peaks for other elements such as molybdenum and aluminum, this suggests the presence of mixed precipitates in *M. oleifera*

plant extracts. This is in agreement with the work of Adebayo-Tayo *et al*., (2019) in which the formation of silver nanoparticles was confirmed and observed the presence of other elements in the plant (*Oscillatoria sp.*) extracts used for biosynthesis of AgNPs.

The results of disc diffusion method revealed that *M. oleifera* plant extracts have antibacterial activities against standard strains of *Escherichia coli* (ATCC33849) and *Staphylococcus aureus* (ATCC 25923). Table 4.1 shows inhibitory activities of leaves extracts. Among plant materials, leaves extracts showed better antibacterial activities with reference to inhibition zones than seeds and roots. This may be attributed to abundant amounts of bioactive compounds in leaves that aid in biological activities than other plant materials. In addition, leaves have soft tissues compared to seeds and roots, this means that phytochemicals that aid in inhibitory activities may be easily extractable. These results are in alignment with Etejere *et al*., (2015) who carried out a quantitative analysis of phytochemical ingredients in different plant parts of *M. oleifera* and found that they are significantly highest in leaves. For this reason, it was deduced that this could be the reason why leaves have been reported to possess various biological activities than other plant parts.

Within leaves extracts, it was observed that ethanol extracts have enhanced inhibitory activities than methanol and aqueous extracts. This might mean that ethanol extracted large amounts of phytochemical constituents than methanol and aqueous. Similar results were reported by Singh & Tafida (2014) who found that ethanol extracts of *M. oleifera* leaves have better antibacterial activities than methanol, aqueous extracts and an antibiotic called septrin which was used as positive control. In the study, it was emphasized that although the traditional method of using the plant as a herb is by boiling it in water, ethanol extracts has

better antibacterial activities. There was a statistically significant difference in antibacterial activities between organic and aqueous extracts on test strains, with organic extracts displaying better inhibitory activities.

Table 4.2 shows inhibitory activities of seed extracts, which was found to possess improved antibacterial activities than root extracts. This may be reflected on the variation in concentration of bioactive compounds in plant parts. More phytochemicals might be present in seeds as compared to roots. In addition, seeds may have active compounds that are not present in roots which may contribute to observed differences in the results. This is in line with Paikira *et al.*, (2017). Several other phytochemical constituents are presence in seeds but not in roots. For instance, lectin, pterygospermin, methionine, niazimicin and niazirin was found to be absent in roots (Paikira *et al*., 2017), this may aid in seed's biological activities. Same as leaves extracts, organic extracts of seeds had a statistically significant difference with improved antibacterial activities compared to aqueous extracts.

As similarly observed in table 4.1 and 4.2, root extracts in table 4.3 also showed a concentration-dependent inhibitory activity. However, the lowest concentration of ethanol extracts showed no inhibition zone. This can be explained by the fact that at 100 mg/mL of root extracts, the concentration of active ingredients were not sufficient enough to inhibit the growth of test organisms. The results are similar to Raj *et al*., (2011) in which it was found that at lowest concentration (5 mg/mL) of ethanol and petroleum ether of *M. oleifera* root extracts, no inhibition zones were shown against *Proteus mirabilis*. As previously observed with leaves and seeds extracts, organic root extracts also showed a significant difference with improved inhibitory activities compared to aqueous extracts. However, the antibacterial activities of roots were significantly less as compared to both plant materials.

With all plant extracts, it was observed that the inhibitory activities decrease as the concentration of extracts decreases, this signifies a concentration-dependent inhibition effect and a direct correlation between concentration of extracts and inhibitory activities. These results can be explained by the fact that as concentration of extracts decreases, the concentration of phytochemical constituents also decreases, resulting in less-and-less active ingredients that acts upon the growth of test organisms. These results are in alignment with previous studies. For example, Amanze *et al*., (2020) evaluated antibacterial activities of root bark extracts of *M. oleifera* on *S. aureus* and *E. coli* where it was found that methanol extracts has better antibacterial activities than aqueous extracts and suggested that this may be attributed to the ability of methanol to extract more essential secondary metabolites that aqueous. However, they observed concentration-dependent inhibitory activities with each solvent used.

It is worth noting that the major similarity observed among *M. oleifera* extracts is that *E. coli* was less inhibited as compared to *S. aureus*. This may be due to their differences in cell walls, *E. coli* has an outer membrane that covers peptidoglycan and serves as a barrier to antibacterial agents, which may not allow sufficient amounts of inhibitory agents to enter the cell. On the other hand, *S. aureus* lacks the outer membrane, leaving the peptidoglycan exposed to surrounding substances thus increasing its sensitivity to antibacterial agents (Das et al., 2017). The findings are in contrast with Bello & Jamiu (2017), the results showed that *S. aureus* was less inhibited by extracts and standard antibiotics than *E. coli*. It was then suggested that this may be due to the production of an enzyme called beta-lactamase (β-lactamase) which hydrolyses β-lactam ring of the antibiotic, resulting in inactivation of β-lactam antibiotics.

All extracts mediated AgNPs showed antibacterial activities against antibiotic resistant strains of *E. coli* (ATCC 35218) and *S. aureus* (ATCC 12489). In table 4.6, leaves mediated AgNPs showed better inhibition zones than seeds and root mediated AgNPs. This lies in the differences of phytochemical constituents present, leaves have more bioactive ingredients than seeds and roots. Hence, AgNPs of leaves may have be capped with phytochemical compounds present in all other plant parts and those unique to leaves only, resulting in improved inhibitory effects. This is in line with earlier reports. Tannins, saponins, alkaloids, cardiac glycosides and flavonoids are all present in leaves and seeds. However, in exception of tannins that are slightly abundant in both plant parts, alkaloids, cardiac glycosides and flavonoids are highly abundant in leaves than seeds (Garga *et al.,* 2019). In addition, Raj *et al*., (2011) reported the absence of tannins in roots of *M. oleifera* and a slight presence of alkaloids and cardiac glycosides. Moreover, the size of nanoparticles also plays a role in inhibiting the growth of bacteria and since they varied with different plant extracts, leaves extracts may have capped with small-sized AgNPs as compared to seeds and roots. This is in line with previous study of Anigol *et al*., (2017) which stated that small-sized AgNPs have better antibacterial activities than large-sized AgNPs.

Furthermore, it was shown that ethanol extracts of leaves mediated AgNPs have the highest inhibition zone, which was acquired against the gram positive strain. Nevertheless, it was smaller than the zones of inhibition attained by streptomycin. It is suggested that this may be due to the infiltration capacity of AgNPs into the cell. In comparison to AgNPs, greater concentration of streptomycin may have penetrated the microbes' cells to cause cell damage. The results collaborate with Adil *et al*., (2019) who found that ciprofloxacin has better antibacterial activities than callus extracts mediated AgNPs.

In table 4.7 of seed extracts mediated AgNPs, the highest ZOI was larger than that of root extracts mediated AgNPs but smaller than that expressed by leaf extracts mediated AgNPs. The difference in inhibitory activity may be sorely based on firmness of AgNPs' incorporation with extracts and bioactive compounds. Although biological activities of *M. oleifera* seeds may be due to an array of phytochemical compounds, a short polypeptide called benzyl-isothiocyanate found in seeds also contributes to the antibacterial activities. This polypeptide inhibits growth of bacteria by disrupting the synthesis of cell membrane and essential enzymes (Burkar *et al*., 2010). This suggests that the coupled action of the polypeptide present and AgNPs may have resulted in greater inhibitory activities in seeds as compared to roots. In the same way with AgNPs of leaves, seed extracts mediated AgNPs obtained ZOI smaller than that of streptomycin which indicates than the antibiotic had better inhibitory activities. In contrast to AgNPs of leaves, methanol extracts of seed-AgNPs presented the highest zone of inhibition. However, the similarity lies in the fact that they are both organic solvents with the ability to extract much more secondary metabolites than aqueous extracts.

Similar observations were noted with root extracts mediated AgNPs in table 4.8. AgNPs of methanol extracts had uppermost inhibition zone, however, this was smaller than the ZOI presented by the positive control, leaves and seed extracts mediated AgNPs. This could be because of the limit of root-AgNPs to hold fast to the bacterial cell layer at high fixations and enter quickly into the cytoplasm which create basic changes in the cell wall and subsequently the inhibition of the organism (Ahmed *et al*., 2016). Overall, it was observed that organic extracts-AgNPs have improved antibacterial activities than aqueous extracts. This can be explained by the fact that during plant extraction, organic solvents extract much

more bioactive compounds from the plant than aqueous solvent. These results collaborate with Amabye & Tadesse (2016) with findings that ethanol leaf extracts of *M. oleifera* have tannins in addition to all phytochemicals present in aqueous extracts.

Moreover, several literatures have reported that ethanol and methanol extracts have high levels of tannins, saponins and flavonoids than aqueous extracts (Garga *et al*., 2019; Shoba *et al*., 2014). In the current study, it was shown that AgNPs of plant extracts have enhanced antibacterial activities than crude extracts only due to the combined activities of secondary metabolites of the plant and small-sized AgNPs that facilitate infiltration into microbes' cells, causing cell death. Islam *et al*., (2021) tested similar doses of AgNPs and crude extracts of *M. oleifera* on several strains and found that inhibitory activities of AgNPs were improved compared to crude extracts.

The combination of AgNPs with antibiotics showed improved antibacterial activities as compared to *M. oleifera* extracts mediated AgNPs. This is due to the presence of the antibiotic that works in association with nanoparticles. Table 4.9-4.11 shows the effectiveness of the association of *M. oleifera* mediated AgNPs with penicillin G. Similar to crude extracts and extracts mediated AgNPs, it was shown that leaves have uppermost activities as compared to seeds and roots. It was suggested that the abundant amounts of secondary metabolites in leaves played a role as capping agents and acted as a bridge for AgNPs and penicillin G into the microbes' cells, leading to cell lysis. This is in line with Mohsen *et al*., (2020) that demonstrated that combination of ciprofloxacin with citrate mediated AgNPs have better antibacterial activities than ciprofloxacin alone and AgNPs alone, and suggested that it was due to citrate that acted as a bridge of AgNPs and ciprofloxacin into bacterial cells.

As observed in table 4.9, the combination of ethanolic seed extracts with penicillin G in table 4.10 also presented the highest inhibition zone against *S. aureus*. This means that ethanol extracted sufficient secondary metabolites that interacted with AgNPs and penicillin G, resulting in improved antibacterial activities as compared to methanol and aqueous extracts. However, the highest ZOI was smaller than that obtained with leaves extracts and larger than that of roots extracts. These differences can be explained by the availability of bioactive ingredients in different plant parts as well as sizes of AgNPs, AgNPs synthesized from seeds extracts may have been smaller than those from roots extracts.

Equally important, it was also observed that the association of AgNPs with the antibiotic has better antibacterial activities than streptomycin alone and AgNPs alone. This may be due to the fact that AgNPs attach to the cell membrane and induce pit formation through which antibiotics and nanoparticles penetrate through into bacterial cells at high concentrations, causing intracellular damage. The coupled actions of nanoparticles, secondary metabolites and penicillin G on and within the bacterial cell might have led to the enhanced antibacterial activities. This is in alignment with the study of Adil *et al*., (2019) in which it was found that the association of AgNPs and ciprofloxacin has better antibacterial activities than ciprofloxacin alone.

In table 4.11, the combination of root extracts mediated AgNPs with penicillin G also showed findings similar to the combination of the antibiotic with leaves and seeds mediated AgNPs. Among solvents, organic extracts showed enhanced antibacterial properties than aqueous extracts. The results are in correlation with Dewangan *et al*., (2010) in which it was observed that root bark aqueous extracts of *M. oleifera* have minimum antibacterial activities as compared to all organic extracts used against *E. coli, S. aureus, P. aeruginosa* and

Staphylococcus gallinarum. The fact that roots inhibit the growth of bacteria less than leaves and seeds may strongly be attributed to the presence of less biomolecules that interact with AgNPs and penicillin G, resulting in minimum activities.

Tables 4.12-4.14 shows inhibitory activities of the combination of AgNPs with streptomycin. Similar to the combinations of *M. oleifera* mediated AgNPs with penicillin G, the association of streptomycin with AgNPs showed improved antibacterial activities as compared to AgNPs only. In addition, the combination had larger inhibition zones than streptomycin alone, thus, streptomycin + *M. oleifera*-AgNPs had better antibacterial activities that the positive control. This is in line with previously reported findings (Lateef *et al*., 2016a; Lateef et al., 2016b).

In all plant materials, organic extracts showed highest zones of inhibition compared to aqueous extracts. Ethanol extracts were mostly efficient than methanol extracts in leaves and root extracts mediated AgNPs + streptomycin. However, the combination of methanol extracts mediated AgNPs + streptomycin showed improved inhibition zones than other extracts in seeds. This is explained by the fact that secondary metabolites are better extractable with organic solvents than aqueous solvent. These results are in agreement with Bello & Jamiu (2017), methanol extracts of seeds have improved antibacterial activities than ethanol and aqueous extracts. It was then suggested that results are linked with the presence of phytochemical constituents in seeds and this coupled with the actions of antibiotics could lead to enhanced antibacterial effects (Bello & Jamiu, 2017).

In the current study, synthesized AgNPs have been observed to possess antibacterial activities against test antibiotic resistant strains of *E. coli* and *S. aureus,* which is similar to previous researchers (Adil *et al*., 2019, Lateef *et al*., 2016a, Gupta *et al*., 2020; Das *et al*.,

2017). However, resistance of microorganisms to biosynthesized AgNPs have been reported by literature. A report evaluated antibacterial activities of silver nanoparticles using seed extracts of *Artocarpus heterophyllus* Lam against some clinical isolates, no inhibition zones against some gram positive and gram negative bacteria were recorded (Jagtap & Bapat, 2013). The resistance could be caused by a compound of bacterial flagellum called flagellin, which makes the nanoparticles clump together and make them lose their efficacy against bacteria (Panacek, *et al*., 2018).

Plant extract mediated AgNPs and in combination with antibiotics were also more effective on *S. aureus* than *E. coli*, this is attributed to their differences in cell walls. *E. coli* is gram negative, it has an outer membrane that does not only serve as a barrier in blocking entry of antibiotics and harmful toxins or enzymes but also covers thin layer of peptidoglycan which is essential in protection of bacteria from environmental stress. The outer membrane can prevent antibacterial agents from reaching the peptidoglycan, preventing cell lysis and cell death. On the other hand, *S. aureus* is gram positive, meaning that it lacks an outer membrane and has an exposed thick layer of peptidoglycan. In this case, peptidoglycan absorbs surrounding materials and antibacterial agents (Das *et al*., 2017). This results in disruption of the cell wall and makes the bacterium more sensitive to antibiotics. This means that *E. coli* was less inhibited as compared to *S. aureus* due to the presence of an outer membrane that increases the impermeability of the cell wall. These results are in contrary with Kambale *et al*., (2020) and Niluxsshun *et al*., (2021).

Kambale *et al*., (2020) found that *E. coli* is more sensitive to AgNPs than *S. aureus* and suggested that the gram negative bacteria are generally more prone to Ag^{+} than gram positive due to their cell wall differences. It was stated that the observed results were due to the fact

that gram negative possess a single layer of peptidoglycan, which makes it easy for Ag^{+} to damage the cell wall. Alternatively, gram positives have a cell wall with thick peptidoglycan layer that serves as a barrier for Ag+ ions penetrating the cytoplasm. Likewise, Niluxsshum *et al*., (2021) found that *E. coli* is more sensitive to AgNPs than *S. aureus* and suggested that it might be due to the differences in composition and thickness of peptidoglycan. It was argued that *S. aureus* possess a three-dimensional peptidoglycan layer almost ten times thicker than the gram negative's peptidoglycan and this results in less susceptibility to AgNPs.

Table 4.17 shows minimum inhibitory concentration of *M. oleifera* leaves extracts. Both bacterial strains obtained equivalent MIC values with each leaf solvent extract. However, organic extracts presented MIC values 2 folds lower than that obtained by aqueous extracts. This may suggest that ethanol and methanol had same concentration of extracts that acted upon bacterial cells that resulted in revealing same MIC values. On the other hand, aqueous may have extracted lower concentration of phytochemicals that had effects on both strains as it has been reported by several literature that secondary metabolites are less extractable with aqueous solvent. These results are in alignment with Fouad *et al*., (2019), in the previous study, I was found that ethanol extracts of *M. oleifera* have same MIC values on *S. aureus* and *E. coli.*

Table 4.18 shows the MIC of seeds extracts. The lowest MIC value was revealed by ethanol extracts on *S. aureus*, this was 2 folds lower than that obtained against *E. coli*, making the gram negative strain less sensitive due to the presence of the outer membrane. These results are in contrast to Bello & Jamiu (2017) who reported methanol seed extracts to have lower MIC value that ethanol and hot aqueous extracts with the suggestion that active compounds

were mostly in methanol extracts. In the current study, methanol seed extracts revealed same MIC values to those observed in leaves methanolic extracts. On the contrary, aqueous extracts had no detection against *E. coli.* Similar results were observed by Fouad *et al.*, (2019) in which hot water extracts have no MIC values on all test organisms used in their study.

Minimum inhibitory concentration of roots extracts is shown in table 4.19. In contrast to leaves and seeds, the lowest MIC value was obtained with methanol extracts against *E. coli*, this was 2 folds lower than the lowest MIC of *S. aureus.* This could be due to the fact that most of the active ingredients were present in methanol extracts. In addition, aqueous extracts showed no detection against any of the bacterial strains and this could be attributed to factors such as the age of the roots used and the extracting solvent as it was suggested by Amanze *et al*., (2019) that the inactivity of the plant can be brought about by several factors such as method of extraction, time of harvest of the plant material, age of the plant and extracting solvent, all of which affects the amount of compounds and chemical composition of the plant. These results are similar to earlier findings found that cold aqueous extracts have no effects (no MIC values) on *S. aureus, E. coli* and *P. aeruginosa* (Bello & Jamiu, 2017). For this reason, it was suggested that aqueous extracts of plants generally exhibit little or no antimicrobial activities because they may have myriads of compounds that may interact antagonistically in their overall activities. Another reason suggested was that active ingredients from plants materials are not readily extractable by water.

Table 4.20 shows MIC of synthesized AgNPs using *M. oleifera*. It was shown that plant-based nanoparticles have effects against both strains. The lowest MIC value was obtained against *S. aureus* by root-based AgNPs, this was two folds lower than that obtained against

E. coli. Aqueous extracts revealed MIC values higher than those of organic solvents. However, ethanol extracts mediated AgNPs were much effective as compared to methanol based AgNPs. Although leaves and seeds have been reported by literature to have abundant amount of phytochemical ingredients than roots, it is suggested that in the current study, roots mediated AgNPs showed the lowest MIC because of firm interaction between phytochemicals compounds and AgNPs. In addition, AgNPs may have bound to microbe's membrane and penetrated into the cell in greater concentration which resulted in greater intracellular damage.

Table 4.21 shows the MIC values obtained by the association of *M. oleifera* mediated AgNPs with penicillin G. In this case, it was revealed that leaves have lowest MIC compared to seeds and roots. This may be probably because leaves have more phytochemical constituents that interact well with AgNPs than in seeds and roots. This is in agreement with earlier research studies, for instance, it was reported that the concentration of phytochemical constituents is significantly higher in leaves than other plant parts (Etejere *et al*., 2015). As similarly observed in table 4.20, organic extracts were more effective based on MIC values compared to aqueous extracts. However, the combination with penicillin G presented the lowest MIC of six folds lower than that observed in table 4.20. The observed difference arises due to the presence of an antibiotic that is carried and delivered by AgNPs into the bacterial cell. This is in line with Ghosh *et al.*, (2012) who stated that in the association of AgNPs with antibiotics, nanoparticles act as a drug carrier by transporting antibiotics to the cell surface where permeability is increased, facilitating antibiotic penetration into the cell. The association increases the concentration of the antibiotic at the bacterial site which results in improved antibacterial activities.

As similarly observed in table 4.21, the combination of *Moringa*-AgNPs with streptomycin in table 4.22 showed that leaves extracts are more efficient than those of seeds and roots. In addition, the lowest MIC value was four folds lower than that presented by *Moringa* mediated AgNPs. However, it was two folds greater than the MIC obtained by combination of *Moringa*-AgNPs with penicillin G. This means that *Moringa*-AgNPs-penicillin G had better antibacterial activities than the combination of streptomycin. This can be explained by partial aggregation of AgNPs observed. The aggregated AgNPs combined with streptomycin may have hindered the infiltration of the antibiotic into the microbe's cell, resulting in less surface area and contact with intracellular structures which in turn leads to reduced inhibition activities. Similar findings were reported by Mohsen *et al*., (2020) who observed that the combination of AgNPs with ciprofloxacin have less antibacterial activities than ciprofloxacin alone and stated that it might be due to agglomeration of silver nanoparticles. It is worth noting that AgNPs showed inhibitory activities with and without association of antibiotics, however there is variation in MIC values and this is due to the different concentration of bioactive ingredients present in different plant parts.

The association of *Moringa*-AgNPs with antibiotics produced lower MIC values and better antibacterial activities than *Moringa*-AgNPs only. It is suggested that AgNPs may have bound to sulfur-containing proteins which increased the permeability of the membrane. After, they penetrated into the cell where they produced reactive oxygen species that damaged the cell's internal structures (Applerot *et al*., 2012). In addition, AgNPs may have caused depletion of adenosine triphosphate (ATP) production and disruption of deoxyribonucleic acid (DNA) replication (Rai *et al*., 2009). It is further suggested that the combination of *Moringa*-AgNPs with antibiotics is more powerful and have better inhibitory

activities because if the bacteria is less affected by one of the agents, another antibacterial agent would inhibit the growth of the strain in a different way. The joined action caused more cell damage than *Moringa*-AgNPs. This is similar to Wan *et al*., (2016) who found that the combination of AgNPs with antibiotics (polymyxin B, rifampicin and tigecycline) increases survival rates in *Acinetobacter baumannii*-infected mouse peritonitis models than AgNPs and antibiotics alone. The combination of AgNPs with antibiotics showed enhanced zones of inhibition compared to AgNPs alone and streptomycin alone due to nanoparticles that increase the concentration of antibiotics at bacterium-antibiotic site, facilitating great penetration into the cells (Hassan *et al*., 2016).

6 Conclusion

The current study demonstrated an eco-friendly, inexpensive and fast method of synthesizing silver nanoparticles from *M. oleifera* using methanol, ethanol and aqueous extracts specifically from leaves, seeds and roots. The successful formation of AgNPs were confirmed by several techniques such as UV-vis spectroscopy, fourier transform infrared spectroscopy and scanning electron microscopy. Phytochemical constituents have a key role in antibacterial activities of the plant as well as synthesis of AgNPs. The biological assessment of AgNPs revealed good antibacterial activities against antibiotic resistant strains of *S. aureus* and *E. coli*.

It can be concluded that leaves are most effective as compared to seeds and roots. Among solvents, ethanol is the best extracting solvent for antibacterial activities of *M. oleifera* against *S. aureus* and *E. coli*. Since antibacterial activity decreased with decreasing concentration of extracts, it was concluded that there was a concentration-dependent

inhibition on both bacteria. This study showed that all plant materials and extracts have antimicrobial activities against test microorganisms, this can suggest that *M. oleifera* can be a source of antimicrobial agents with potential in controlling diseases associated with infections caused by these pathogens. In this case, the combination of AgNPs with antibiotics showed better activities than AgNPs alone and streptomycin alone, thus this study attested that AgNPs synthesized via green method using *M. oleifera* medicinal plant and in combination with common antibiotic approaches sheds light in the direction of decreasing the odds of antibiotic resistance development of these pathogens.

7 Recommendations

Firstly, further studies are required to assess Minimum Bactericidal Concentrations (MBC) of the AgNPs of *M. oleifera* extracts. Secondly, additional studies are required to combine streptomycin and penicillin G together with as-prepared AgNPs for antibacterial activity evaluation. Moreover, further studies are needed to assess possible side effects that may arise in using the combination of AgNPs and common antibiotics used in this study as antibacterial agents.

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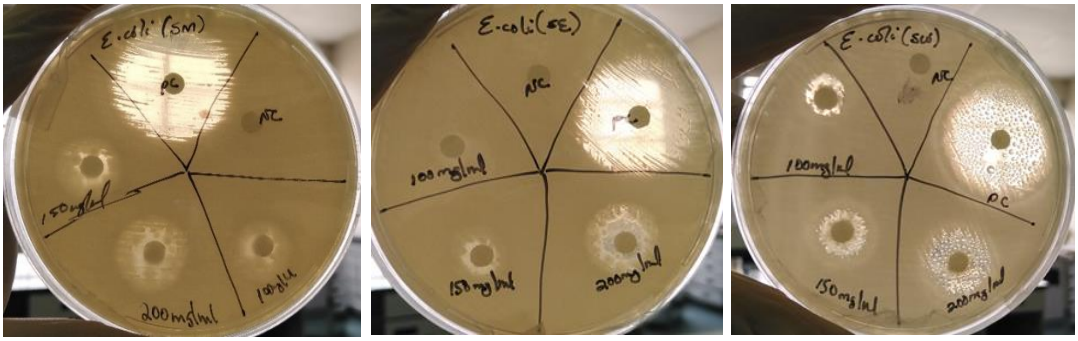
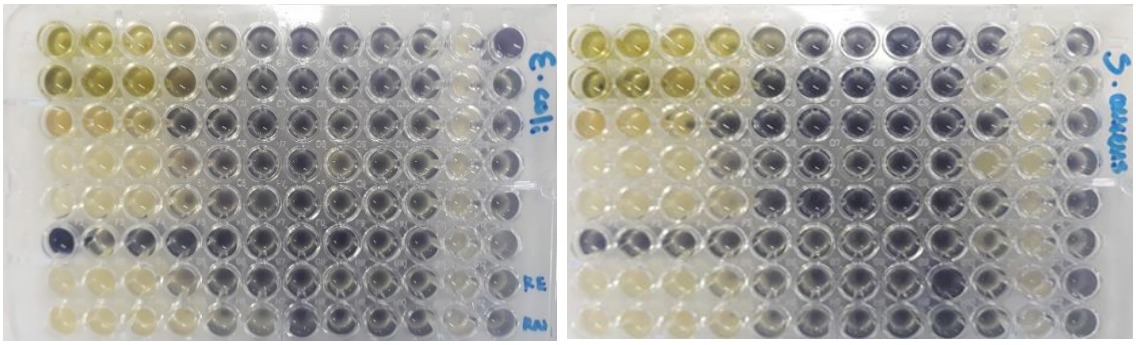
Appendices:

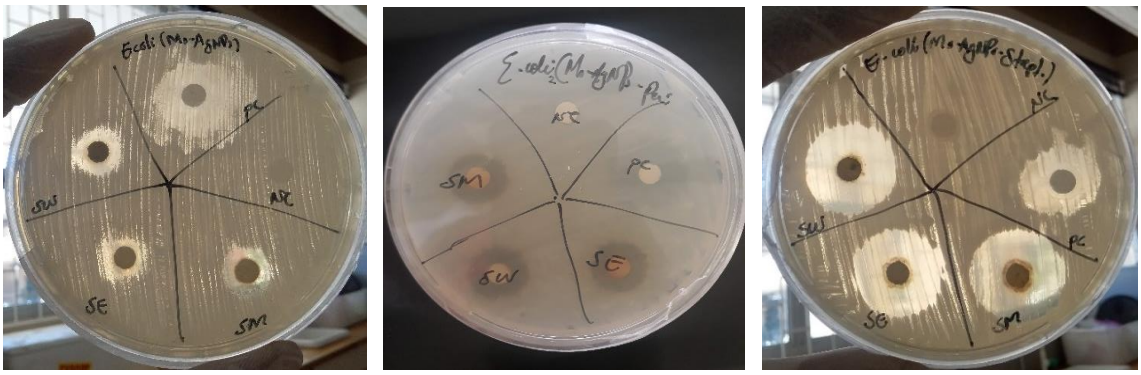
Petri plates for antibacterial activities of plain extracts.



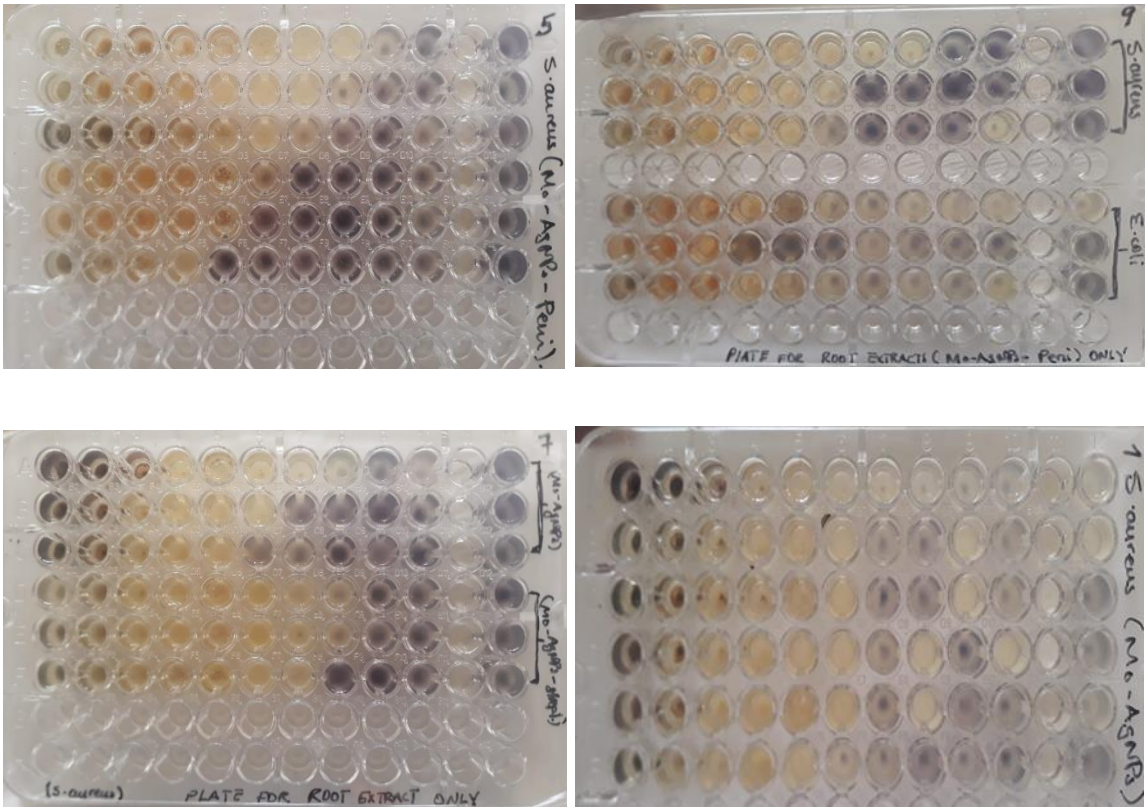
Images of Minimum inhibitory concentrations of plain extracts.

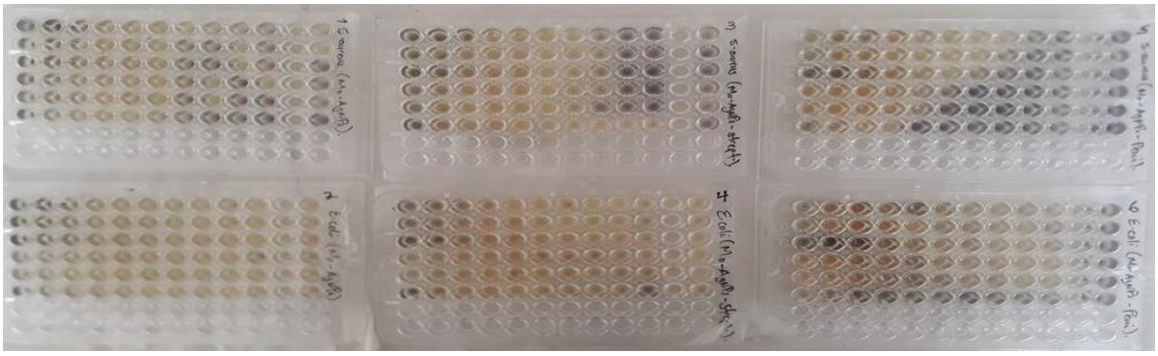


Petri plates for antibacterial activities of AgNPs.

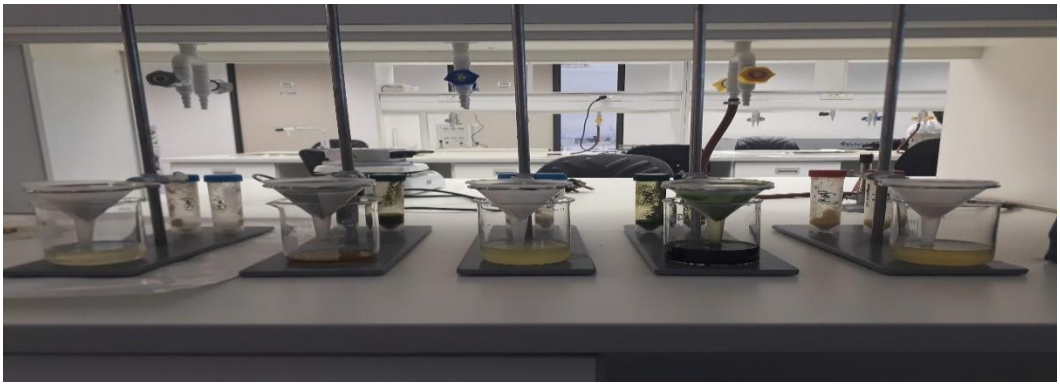


Images of Minimum inhibitory Concenturation of AgNPs.





Materials used:



Filtration of plants extracts.

Rotary evaporator

Streptomycin, Penicillin G and Silver nitrate pellets; UV-1600PC Spectrophotometer