https://dx.doi.org/10.4314/dujopas.v9i4b.18
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 4b December 2023*

Synthesis of AGNPs from the Seed oil of *Terminalia mantaly* and its Antimicrobial activities against selected Bacteria

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Abstract

Antibiotic resistance is a global public health problem that requires innovative alternatives to reduce the loss of life. This study investigated the antibacterial activity of T. mantaly seed oil and synthesized silver nanoparticles (AgNPs) on antibiotic resistant bacterial strains. The seed oil was extracted by the Soxhlet method using n-hexane and petroleum ether and characterised by GC-MS and FTIR analysis. The AgNPs were investigated using a UV-vis spectrophotometer and the antibacterial potential of the oils and AgNPs was evaluated using the agar well diffusion method. The eighteen (18) compounds identified were phenolic esters with 9, 12-octadecadienoic acid (Z, Z), eicosenoic acid and 9-octadecanoic acid being the most prominent in the oils. 3-Hexen-1-ol, diethyl phthalate and 1, 2-benzene dicarboxylic acid were found for the first time in T. mantaly seed oil. AgNPs showed 26.83±3.01 mm activity higher than 19.83±1.89 mm of seed oil with no resistance shown by the test bacteria. While K. pneumoniae and S. aureus showed selective resistance to the oil. The study revealed the potency of AgNPs synthesized from T. mantaly seed oil against antibiotic-resistant bacterial strains due to the presence of novel compounds.

Keywords: Antibacterial activity, Antibiotic resistance Bacteria, Bioactive compounds, Silver nanoparticles, Terminalia seed oil,

INTRODUCTION

Antibiotic resistance is a global health problem that claimed 1.2 million lives in 2019 (Tang *et al*. 2023; Founou *et al*. 2021). This drives the search for new medical alternatives to limit future occurrences and damages. Nanoparticles are new antimicrobial agents that have shown unique potential against antibiotic resistance. The production of different biocompatible sizes (1-100 nm) from different sources using physical, chemical and biological methods expands the manufacturing techniques. However, the biological method is a cost-effective and safe route that will increase their availability for medical applications (Hasan *et al*. 2022). Nanoparticles increase the efficacy of herbal medicines due to their high surface area-to-

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volume ratio, which rapidly destroys microbial cells (Pasupuleti et al., 2008; Majoumouo *et al*. 2020). In addition, free radicals on their molecules increase cell damage by increasing oxidative stress and protecting human tissues from the effects (Lee et al., 2006; Sidhu *et al*. 2022).

Plant products are sources of bioactive components with lethal effects on pathogenic microorganisms (Lopez-Romero *et al.* 2015). These natural products have been used to save a larger population of rural Nigerians from common diseases due to the high cost of drugs (Magrani *et al*. 2005). Thus, science in traditional medicine can make a significant contribution to improving access to medicines and achieving universal health coverage in Africa (WHO, 2019). Furthermore, drug resistance requires innovative alternatives to interrupt transmission during infections (Ahmed et al., 2015; Hunter, 2001). Essential oils have the potential to cure diseases, but limited therapeutic efficacy has compromised their medicinal value. The synthesis of AgNPs from essential oils represents a new strategy that will enhance their antibacterial efficacy and medical utility in the treatment of diseases (Selvakumar *et al.* 2023). Terminalia spp are medium to large fruit and nut-producing trees that are widely distributed throughout the world. Of the various species found in Nigeria, *Terminalia mantaly* has been used by traditional healers to treat various diseases such as tuberculosis, cholera, malaria, typhoid, hepatitis, stomach ache, diabetes, diarrhoea, gonorrhoea and wounds (Iheagwam *et al.* 2021). In addition, the plant has traditionally shown potency in the treatment of diseases caused by well-known multidrug-resistant strains such as *E. coli, K. pneumonia, S. aureus, S. typhi* and *S. pyogenes* (Korzeniewska et al. 2013; Moiketsi et al. 2023). For example, ethanol leaf extract was reported to have antimicrobial activity against *S. pneumonia, S. aureus, E. coli, A. niger* and *C. albicans* comparable to gentamicin and ketoconazole drugs (Ebele *et al.* 2021). While Mbosso *et al.* (2021) found that methanol and hexane root extracts inhibited *S. aureus* and *Trypanosoma brucei brucei*. In another study, Samuel and Adekunle (2021) found methanol extract of the stem bark to be effective in treating malaria parasites. This potential attributed to its synthetic natural products for self-defense (Ebele *et al*. 2021), stimulated interest in elucidating the pharmacological properties of different parts of the plant.

Therefore, this study aimed to investigate the antibacterial effects of *T. mantaly* seed oils and identify their active chemical compounds. In order to further explore the therapeutic benefits, the seed oils were modified with $AgNO_3$ and screened for antibacterial effects against pathogenic bacterial strains. The AgNPs give adequate protection to human tissues and produce surface ions that rapidly combat disease-causing microorganisms. Due to their non-toxic properties, they are widely used in the medical, food and water industries and have led to higher crop yields in agriculture. These unique properties make AgNPs an alternative to the increasing bacteria resistance and ineffectiveness of antibiotics. The application of AgNPs is therefore expected to advance research into their properties and improve our lives.

MATERIALS AND METHODS

Area of Study

The study site is situated at the Bosso Campus of the Federal University of Technology, in Niger, Nigeria (Lat 9.654626º long 6.530143º). It is positioned directly after the Bosso Pedestal Bridge, when approaching from the town, and is surrounded by *Terminalia mantaly* trees that provide shade across the area.

Collection of Sample

Terminalia mantaly fruits (2500g) were collected from trees at the Federal University of Technology, Bosso Campus, Minna. A fresh branch of the plant containing leaves and fruit

was used for authentication in the Department of Biological Science IBB University, Lapai. The fresh fruits collected in a polythene bag were rinsed in distilled water and spread on a clean leather to dry in an open space in the Microbiology laboratory, IBB University, Lapai, Niger, Nigeria. After drying, the sample was crushed in a cleaned mortar and pistil to expose the seeds endosperm for extraction of the oils (Olaniyan and Yusuf, 2012).

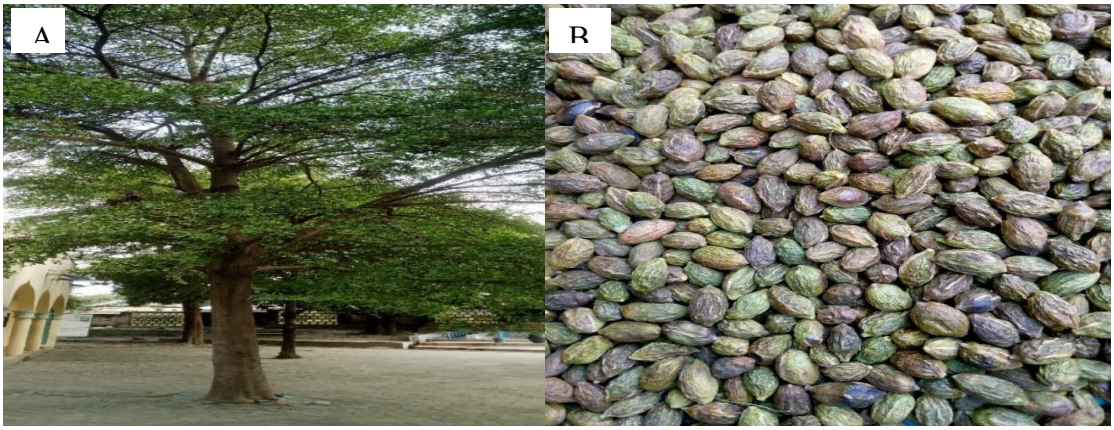

Figure 1: A; typical *Terminalia mantaly* plant and B; the fruits the seeds of the plant

Study design

The detailed step-by-step experimental design for this study is diagrammatically shown in Figure 2.

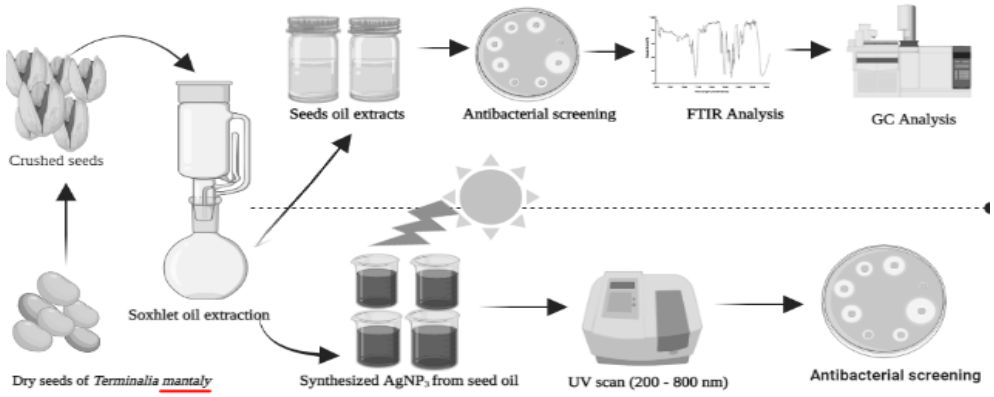

Figure 2: Experimental design for the study

Soxhlet Extraction of Oils

The oil was extracted according to the method of Dessy et al. (2019) using n-hexane and petroleum ether. Although extraction was performed several times, a total of 1356 g and 1520 g of crushed seeds were extracted with n-hexane and petroleum ether using a Soxhlet extractor and heated to 65°C. After extraction, the oil was filtered through Whatman No.1 and the solvent evaporated in a hot water bath.

Identification of Oil Compounds by GC-MS Analysis

The identification of the oil compounds was carried out by GC-MS analysis as described by Awa et al. (2012). One mL of the diluted oil was analysed by GC-MS using an Agilent Split/Splitless and a BP5 (30m) equipped with a (0.25m 0.25m) capillary column (30m 0.25m 0.25m). Nitrogen was the carrier gas and the column was held at 60°C for 3 minutes, ramped to 220°C for 5 minutes and then held constant at 220°C. The temperature interface was set at 280°C. Mass spectrometric analysis gave 40,800 atomic mass units (amu) at 2,500°C. The

identified compounds have peaks with greater than 90% accuracy compared to more than 62,000 spectral samples in the National Institute of Standard and Technology (NIST) library database and Wiley libraries.

FTIR Analysis of Oils

Fourier Transform Infrared Spectroscopy was used to identify the functional group of *Terminalia mantaly* seed oils and to identify closely related compounds. Samples were applied to the clean window of the Cary 630 using Agilent technology and Diamond ATR (Attenuated Total Reflectance). The pressure clamp was then closed until a click was heard and analysed using Micro-Lab real-time software at wavelengths between 4000 and 650 cm-1 (Paharudin *et al.* 2022).

Synthesis of AgNPs

The extracted oils were treated with silver nitrate solution ($AgNO_3$) using a modified method of Chandran *et al*. (2006). A 0.07 g of $AgNO_3$ was dissolved in 200 ml of distilled water to make a 2 mM stock solution. Then 10 ml of $AgNO_3$ was added to 4 separate beakers containing 5, 2.5, 1.5 and 0.625 ml of the oil to give $AgNO_3$-oil ratios of 1/2, 1/4, 1/8 and 1/10 and shaken gradually. The reaction was enhanced by incubating the mixture at 37°C for 30 minutes in daylight. The solutions were scanned in a Shimadzu UV-Vis spectrophotometer at wavelengths of 200 and 800 nm to confirm the formation of AgNPs.

Antimicrobial screening of seed oils and AgNPs

The antimicrobial tests were carried out using the agar well diffusion method (Nauman and Arshad, 2011). The oils and AgNPs were screened against five bacterial strains (*E. coli, K. pneumoniae, S. aureus, S. typhi* and *S. pyogenes*). Before screening, 1.3, 1.0, 0.7 and 0.4 ml of the oils dispensed into beakers were diluted with 3.7, 4.0, 4.3 and 4.6 ml DMSO to give 6, 14, 20 and 26% oils. DMSO was chosen because the solvent has no antibacterial activity. Four wells were drilled under aseptic conditions using a 6 mm cork borer on fresh nutrient agar plates and inoculated with bacteria. A 0.2 ml of oil was added to the wells and after 24 hours of incubation at 37°C, the inhibition zones were examined. The same procedure was followed without dilution with DMSO for the silver nanoparticles. In control experiments, 0.2 ml of 100 mg/ml Ampiclox was tested against each bacterial strain using the same procedure.

Statistical Analysis

One-way ANOVA statistical tools with post-hoc-Duncan alpha at a significance of 0.05 were used to analyze the experimental results obtained

RESULTS AND DISCUSSIONS

A viscous yellowish oil was extracted from the seeds using n-hexane and petroleum ether. The reaction of $AgNO_3$ with oils resulted in a light brown colour. The UV-vis peaks for the synthesised AgNPs are shown in Figure 2. The peaks at 1/2, 1/3 and 1/8 dilution correspond to 300-350 nm wavelength. However, a peak at 230 and 310 nm was also observed in the spectra for 1/10 oil diluent. The absorbance decreased with increasing oil concentration from 1/2 to 1/10 oil dilutions. This shows that the oil concentration influences the reaction and formation of AgNPs (Gonfa et al. 2023). The brown colour change indicated the formation of AgNPs, which is consistent with the findings of Deepak et al. (2017). Such colour changes depicted the excitation of the plasmon at the surface of the synthesized AgNPs, similar to the observation of Awad et al. (2021), confirming our results. In addition, the heating temperature and time resulted in the production of AgNPs at lower wavelengths, different from the usually reported 300 to 450 nm (Abdel-Aziz et al., 2014; Bharathi et al., 2018). AgNPs have

been described to be synthesised in different particle sizes, but the smaller-sized species are more reactive due to increased antibacterial efficiency.

Compounds Identified by GC-MS Analysis

Figure 3 shows the GC-MS results of the chemical compounds identified in the seed oil. Eighteen (18) similar compounds were identified in the 2 oil extracts. Among the eighteen (18) compounds identified in this study, 9, 12-octadecadienoic acid (Z, Z) (19.6%), eicosenoic acid (8.7%) and 9-octadecanoic acid (7.4%) were the most prominent compounds. The chromatogram peaks of the identified compounds are shown in Figure 3. The compounds showed the same weight percentage, except for ecosanoic acid, which was extracted more by n-hexane (Fig. 4). Most of the compounds were present in low concentrations with values not exceeding 5.45%. 3-Hexen-1-ol, diethyl phthalate is a methyl ester, while diethyl phthalate and 1, 2-benzene dicarboxylic acid are the only diethyl and diisooctyl esters found in the oils, suggesting new compounds isolated for the first time in *T. mantaly* seed oil. Moderate antibacterial activity of 1, 2-benzene dicarboxylic acid isolated from *Nauclea latifolia* against *B. subtilis* and *S. aureus* was detected by Fadipe et al. (2014). Diethyl phthalate, previously isolated from the leaves of *Andrographis paniculata*, was also reported to have inhibitory activity against *S. aureus* and *E. coli* (Navis et al. 2022). This is consistent with previous findings of the inhibitory effects of these compounds on Gram-positive and Gram-negative pathogens. Therefore, the efficacy of the oil on the bacterial strains studied must have been due to the synergy of these compounds. The different phenolic esters identified by GC-MS analysis were consistent with the functional groups reported by Rexhepia et al. (2019).

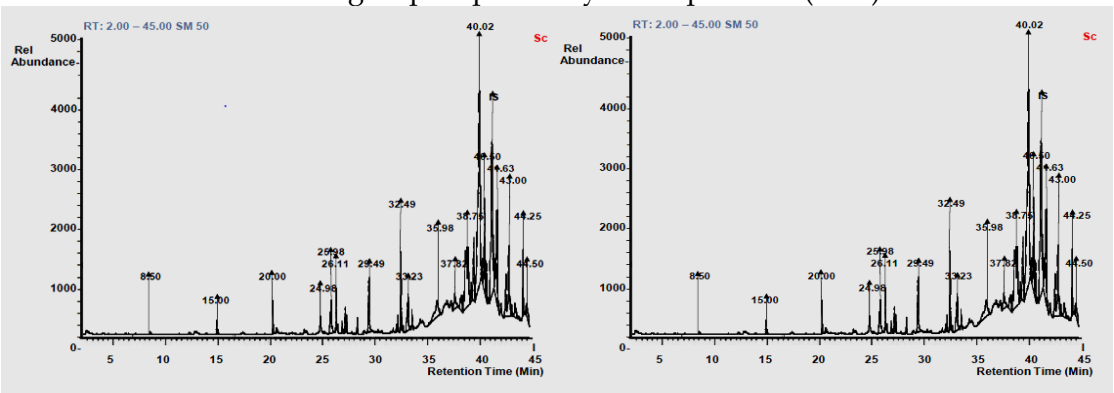

Fig. 3: The GC-MS peaks that identified chemical compounds in the seed oil extracted with n-hexane and petroleum ether

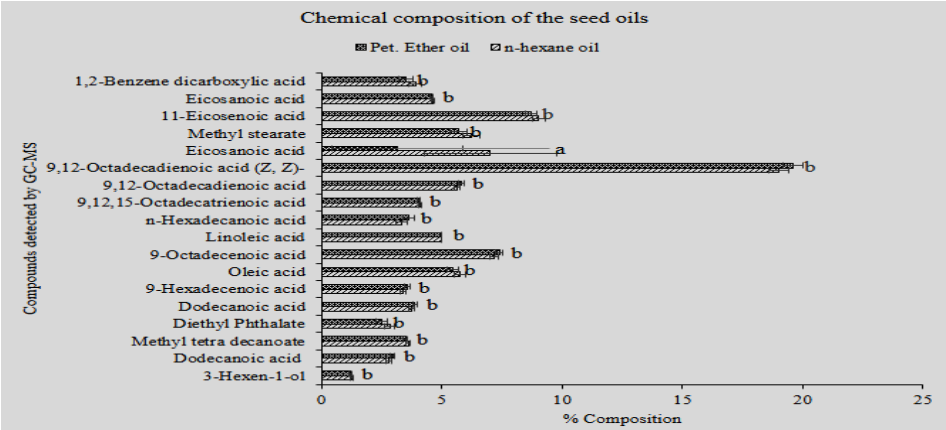

Fig. 4: Chemical compounds identified in the *T. mantaly* seed oil and their composition

Table 1 compares the chemical compounds of Terminalia spp seed oil in different studies. The 18 compounds in this study differ from the 8 compounds identified in *T. catappa* from

Ketapang, even when the same solvent was used for oil extraction. Similarly, the compounds in this study are higher than those of *T. catappa* grown in Ibadan, Nigeria (Table 2), indicating different compounds from the growing areas. The chemical constituents of a particular soil and climatic condition determine the phenolic compounds that could be synthesized for plant usage (Allison *et al*. 2013; Adeleke and Babalola, 2020). In addition, the polarity of the solvent used in extraction affects the number of phenolic compounds extracted from seeds (Ghasemzadeh et al., 2011). In this study, the same compounds obtained could be related to the polarity of the 2 solvents (non-polar solvents) used in the extraction process. While 3-hexen-1-ol, 12-octadecadienoic acid (Z, Z) and n-hexadecanoic acid are insect attractants produced by the plant to prevent the spread of bacteria and fungi (D'Auria et al., 2007; Fagbemi et al., 2021). They haven't been reported from *T. mantaly* seed oil, including diethyl phthalate. This revealed the presence of novel biomolecules in the seed oils whose structures are shown in Figure 5. Furthermore, 2-benzene dicarboxylic acid has been reported to have antibacterial activity against *S. typhi, E. coli, S. aureus* and *K. pneumonia* (Ani *et al*. 2023).

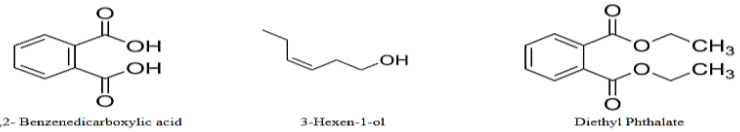

Fig. 5: Structure of new chemical compounds identified in the *T. mantaly* seed oil extract

Table 1 Comparing compounds identified with previous studies on Terminalia seed oils

S/N	This study	*T. chebula* Krishna et al. (2020)	*T. catappa* Ogbeide et al. (2021)	*T. catappa* Napitupulu et al. (2021)	*T. catappa* Pham et al. (2023)
1	3-Hexen-1-ol	Furfural	Cyclopentasiloxane, decamethyl	Hexadecanoic acid	Myristic acid
2	Dodecanoic acid	1,3-Octanediol	Cyclohexasiloxane, dodecamethyl	Octadecanoic acid	Palmitic acid
3	Methyl tetra decanoate	d-Mannose	Cyclopentanetridecanoic acid	9,12-Hexadecadienoic Acid	Palmitoleic acid
4	Diethyl Phthalate	2,4,5-(3H)-Pyrimidinetrione,dihydro-6- hydroxy-	Oleic acid	9,12-octadecadienoic acid	Stearic acid
5	Dodecanoic acid	Carnegine	Dodecanal	10-Octadecenoic Acid	Oleic acid
6	9-Hexadecenoic acid	2-(2-methyl-propenyl)-cyclohexanone	3-hydroxy,dodecanoic acid	9-octadecenoic acid	Linoleic acid
7	Oleic acid	Dodecahydropyrido(1,2-b) isoquinolin-6-one	Cyclooctasiloxane, hexadecamethyl	Docosanoic acid	Arachidic acid
8	9-Octadecenoic acid	Methyl Z-11-tetradecenoate	2-Bromotetradecanoic acid	Tetracosanoic Acid	Linolenic acid
9	Linoleic acid	2-Acetoxy-3,3-dimethyl-2-(3-oxo-but- 1-enyl) Cyclobutane carboxylic	2-bromo, octadecanal	-	cis-11-Eicosenoic acid
10	n-Hexadecanoic acid	Cyclopentaneundecanoic acid	Dodecanal	-	cis-11,14-Eicosadienoic acid
11	9,12,15-Octadecatrienoic acid	Estra-1,3,5(10)-trien-17a-ol	2,9-octadecadienoic acid	-	Behenic acid
12	9,12-Octadecadienoic acid	Oleic acid	n-Hexadecanoic acid	-	-
13	9,12-Octadecadienoic acid (Z, Z)-	Phytol	6,10,14,18,22-Tetracosahexane-3-ol,2,610,15,19,23-hexamethyl	-	-
14	Eicosanoic acid	17-octadecen-1-ol acetate	Hydroxy-3-(1,1-dimethyl prop-2-enyl coumarin	-	-
15	Methyl stearate	But-2-endiamide, N,N′-bis (4-methoxyphenyl)-	10-Octadecanal	-	-
16	11-Eicosenoic acid	Corynan-17-ol,18,19-didehydro-10- methoxy-acetate	9,12-Octadecadienoic acid	-	-
17	Eicosanoic acid	2,4-dimethoxy-10H-acridin-9-one	Phytol	-	-
18	1,2-Benzene dicarboxylic acid	-	9-Octadecenoic acid	-	-
19	-	-	9,12-Octadecadienioc acid	-	-

Key: –; no compound

Table 2 Comparison of the amount of compounds identified by GC-MS analysis in seed oils of Terminalia spp

S/N	Plant Species	Growth area	Oil Source	No. of Compounds	Extraction Solvents
1	*T. catappa*	Vietnam	Seeds	12	Hot and Cold compressed
2	*T. catappa*	Ketapang	Seeds	8	n-hexane
3	*T. chebula*	India	Seeds	17	Ethanol
4	*T. catapa*	Benin, Nigeria	Seeds	15	n-hexane
5	*T. mantaly*	Minna, Nigeria	Seeds	18	n-hexane/Petroleum ether

FTIR Analysis

The FTIR analysis revealed bands at 3008 and 2922 cm-1 corresponding to =C-H, 1742 cm-1 suggesting C=O and 1463 and 1159 cm-1 indicating CH curvature (Fig. 6). These functional

groups represent aldehyde, ester and methyl radicals, confirming the compounds previously identified by GC-MS analysis.

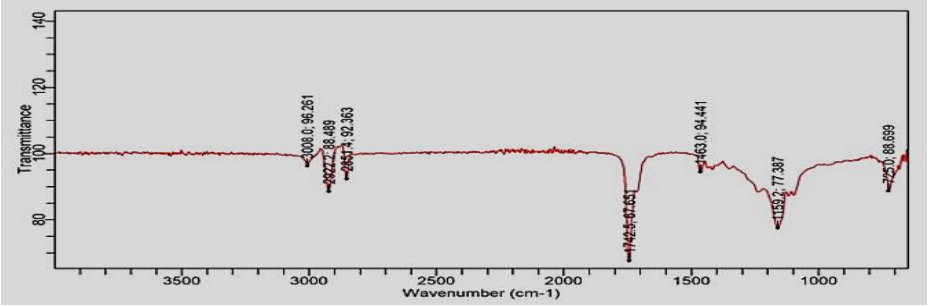

Fig. 6: FTIR spectrum of functional groups in the oil

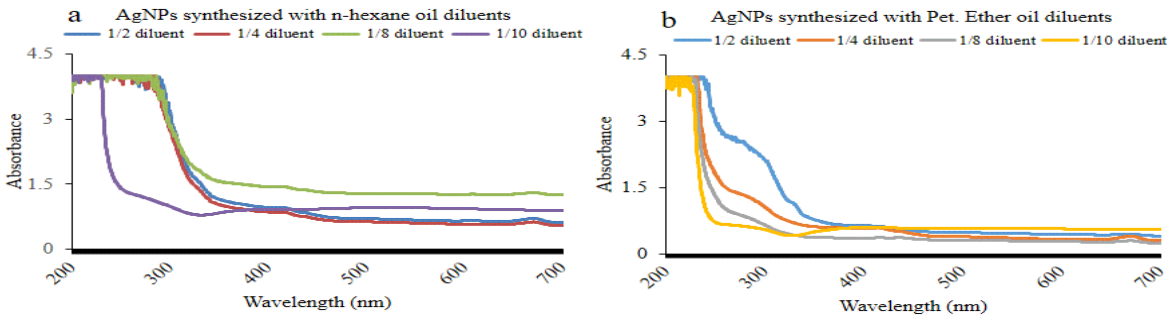

Fig. 6: UV-vis spectra of AgNPs synthesized from *T. mantaly* seed oils

Antimicrobial screening of seed oils and AgNPs

The mean diameters of the clearance zones of the oils/ synthesized AgNPs against the test bacterial strains are shown in Fig. 7. The oil significantly (P>0.05) inhibited the bacterial strains with a higher diameter of 19.83±1.89 mm and 15.91±0.41 mm except for *K. pneumoniae* and *S. aureus* for n-hexane extract. While the lower inhibition zone diameters were 8.66±6.03 mm and 5.58±6.48 mm for *S. aureus* and *K. pneumoniae* for petroleum ether. On the other hand, AgNPs significantly (P>0.05) inhibited all bacterial strains with zone diameters of 26.83±3.01 mm and 19.83±1.89 mm. This shows a strong antibacterial activity in AgNPs than the seed oils. The ability to circumvent the effect of the antibacterial components in the oil may be due to the lipopolysaccharide lignin of the membrane and the development of biofilms. In the case of Gram-negative bacteria in particular, lipopolysaccharides tend to narrow the pores of the membrane to prevent the bioactive molecules from entering the bacterial cells. It is therefore possible that this could be used by *K. pneumoniae* to resist the effects of the seed oil. Biofilm formation is mediated by the gradual development of a thick film around the bacteria. This film protects the bacteria from external attack. Consistent with the findings of Bouchoucha et al (2023), this may have caused the resistance exhibited by S. aureus. Previous exposure to different antibiotics can induce resistance by mutating genes (Jugreet and Mahomoodally 2020; Seid et al 2022). Alternatively, the bacterium can acquire resistance characteristics through horizontal gene transfer (Mohamed et al. 2018). This would render the bioactive oil molecules inaccessible to harmful bacteria. Gram-positive *S. aureus* has been reported to synthesise biofilms to protect cells from foreign molecules (Espina et al. 2015). The slightly higher susceptibility of *S. aureus* than *K. pneumoniae* to seed oil suggests the gradual development of biofilm in the medium. Furthermore, the higher clearance zone of AgNPs shows stronger antibacterial activity than seed oil. Due to the strong activity of their surface ions, researchers reported that the ions rapidly dissolve the membranes and deactivate the genome when they come into contact with the bacteria (Maciel *et al.* 2020; Al-Masoud *et al.* 2021). These ions also disrupt cell permeability and respiration, often due to the strong attraction between the charges on the AgNPs and the membrane (Siritongsuk *et al*. 2016).

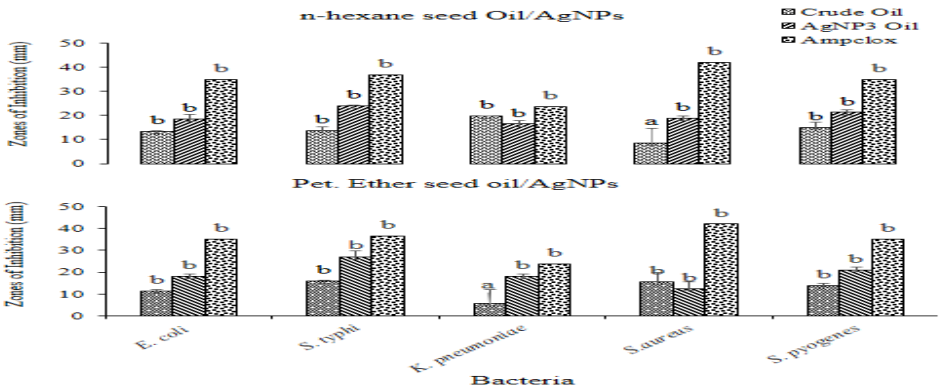

Figure 7: Antibacterial activity of n-hexane and pet. Ether oil extracts *Terminalia mantaly* seeds

The Effect of Dilution on the Overall Zone of Inhibitions

The effect of oil dilution on antibacterial activity is shown in Figure 3. A slight increase was observed in the clearance zones of the different seed oils diluted from 6% to 26% diluent. However, the zone diameter of AgNPs was significantly higher ($P > 0.05$) than that of all the oil diluents, also indicating a stronger antibacterial activity of AgNPs than that of the seed oils.

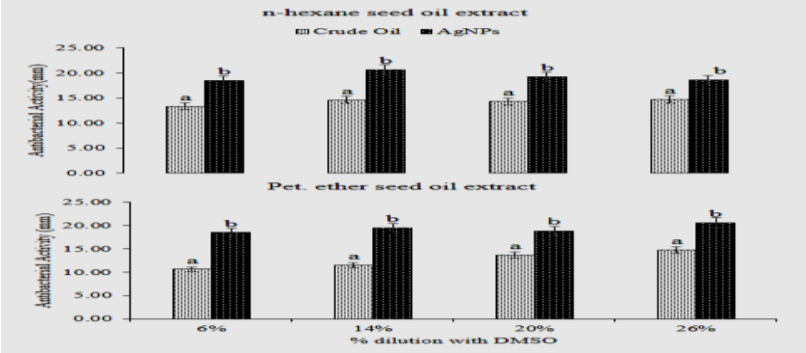

Figure 8: The effect of oil dilution on the bacterial strains inhibition

In general, seed oil and AgNPs showed strong activities, but AgNPs were more effective on the tested bacterial strains. The AgNP's rapid antibacterial activity to all test strains conflicted with the low susceptibility exhibited by *K. pneumoniae* and *S. aureus* to the seed oil, which demonstrated better therapeutic ability. The modification enhances the synergy between novel compounds to damage and kill the bacterium faster at low concentrations.

CONCLUSION

In the present study, AgNPs were synthesized from the seed oil of *T. mantaly*. The characterization of the oil revealed 18 compounds, with 3 compounds identified for the first time. The AgNPs showed significant antimicrobial activity against multidrug-resistant bacteria with selective resistance of *K. pneumoniae* and *S. aureus* to the seed oil. The rapid antibacterial activity of the synthesized AgNPs can be a cost-effective therapy for antibiotic-resistant bacterial strains.

Acknowledgment

We are grateful to Tetfund for providing an IBR grant (Batch 6th 2019 research project intervention) that was used to carry out the experiments in this study. We are also grateful to Mallam Mohammed Hamidu, a senior laboratory technologist at Step B laboratories at the Federal University of Technology Minna, for their efforts in carrying out the sensitive experiments in this study.

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