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Synthesis, Characterization and Antimicrobial Activities of Silver nanoparticles from the Root of *Ficus thonningii*.

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Abstract

This study was carried out to synthesise, characterise and study the antimicrobial activity of silver nanoparticles from the root of Ficus thoningii (Blume). Ficus thoningii were obtained from the Lafia Modern Market, Nasarawa State, Nigeria. Methanolic extracts of root Ficus thoningii were pulverised in the laboratory and extracted with methanol for 30 minutes in an ultrasonicator. The extracts were filtered. Extracts were filtered and reacted with five concentrations of silver nitrate. The formation of the complex was monitored on a UV-visible spectrophotometer for observation of the reaction and absorption peaks. Fourier transform infrared spectroscopy was used to determine functional groups present. The scanning electron microscope was used to determine the shape of the resulting complex. The result showed the formation of AgNPs with a colour change from reddish-brown to a clear brown colour with an absorption peak at about 400 nm. Functional groups identified include ethers, carboxyl, hydroxyl, amines, etc. Bacteria species were highly sensitive to the AgNPs, but fungi were not. This shows that Ficus thoningii root extract formed AgNPs, and the complex had strong antibacterial activity.

Keywords: Silver nanoparticles, Ficus thonningii root extract, Green synthesis, Antimicrobial activity.

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INTRODUCTION

Nanotechnology is an important field in modern science which involves the manipulation of materials at the nanoscale, ranging from 10 to 100 nanometres. They have unique physiochemical properties, such as a large surface area to volume ratio, responsible for their important role in biomedicine, the energy field and other healthcare applications, such as the remarkable antimicrobial properties shown by their potent antimicrobial efficacies against pathogenic organisms (Gavade *et al*., 2015; Ahmed *et al*., 2016; Jalab *et al*., 2021; Abdullahi *et al*., 2024). It represents one of the most significant and active areas of investigation within the broader field of modern materials science, exhibiting novel properties attributable to specific characteristics, including size, morphology and distribution (Altammar, 2023). A great deal of attention has been given to nanotechnology recently as one of the leading interdisciplinary fields, as nanomaterials are often seen as key for a sustainable future due to their physical and technological applications (Malgwi *et al., 2024;* Mohammad and Yusuf, 2024).

Traditional methods for producing nanoparticles, physical (condensation and evaporation) and chemical (oxidation and reduction), have high efficiencies but are costly, involve the use of hazardous chemicals and require significant energy. Researchers have addressed these limitations by developing alternative procedures, which are biological syntheses that are cost-effective, environmentally safe, and user-friendly, utilising plants or microorganisms as reducing and capping agents (Nkosi *et al*., 2024).

Nanoparticles are of metallic and non-metallic types. Metallic nanoparticles have been synthesised from gallium, copper oxide, aluminium, iron oxide, magnesium, gold and silver and are becoming more and more well-known as a result of their physiologically active plant

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secondary metabolites that support their synthesis (Flieger *et al*., 2021). Interest in silver nanoparticles is on the increase due to their chemical stability and other distinctive properties with wide applications as catalytic agents, antibacterial agents or biosensors. Other areas of their applications are anti-inflammatory wound dressing topical creams and antiseptic sprays, as well as cancer diagnosis and treatment (Gavade *et al*., 2015; Jemilugba *et al*., 2019).

Ficus thonningii (*Moraceae*), also known as wild or common wild fig, is an evergreen tree, a native of tropical and subtropical Africa. It grows up to 15 metres tall with a dense, rounded, spreading crown and greyish-brown bark with aerial roots developing from the branches and is widely used across agro-ecological and cultural systems for food, medicine, fodder and ecosystem services (Orwa *et al*., 2009). There has been increasing documentation of its chemical composition, biological activities, role in livestock systems and potential for modern applications such as nanoparticle synthesis in the last two decades (Dangarembizi *et al*., 2012; Balehegn *et al*., 2014; Ondigo *et al*., 2022)**.** Across several African communities, its different parts are used in traditional medicine to address gastrointestinal disorders (e.g., diarrhoea), urinary and respiratory complaints, diabetes, parasitic infections and even certain mental health conditions, with the leaves and stem bark as the commonly used parts (Dangarembizi *et al*., 2012; Gahamanyi *et al*., 2021). In Nigeria, it is known as Chediya (Hausa), Odan (Yoruba) and Odan-abaa (Igbo). It has been reported to contain various bioactive compounds such as alkanoids, terpenoids, flavonoids, tannins and active proteins, which contribute to curative properties (Usman *et al*., 2010).

The root has been reported to give relief from malaria, fever, hepatitis, dental pains, and pneumonia, as well as in the treatment of nose bleeding, stomach pains, chest pains, and diseases caused by evil spirits (Ahur *et al*., 2010; Prelude, 2011).

It has been shown that *F. thonningii* acts as a reducing and capping agent to produce silver nanoparticles (AgNPs) with extracts showing high antibacterial activity (Ondigo *et al*. 2022). The reducing and capping activity guarantees the stability of the nanoparticle (Frieger *et al*., 2021).

Silver nanoparticles have been synthesised from plant extracts of leaves of *Nicotiana tobacum,* neem *(Azadirachta indica*), *Acacia cyanophylla* (leaves, flowers and stems), Matricaria chamomilla, Salvia officinalis, Arabica coffee and Acalypha indica. (Sani *et al*., 2024; Thliza *et al*., 2025) and proven to show the most effective antimicrobial activities from their uniqueness in combating threats and antimicrobial resistances, thereby safeguarding global public health. Their synthesis is approached from "top-down" and "bottom-up", with the latter preferred because it starts from simpler particles to clusters and proceeds to nanoparticles. Synthesis methods for nanoparticles can be physical, chemical or biological in approach, with the biological overcoming most of the drawbacks encountered in the physical and chemical approaches through the use of biological agents such as yeasts, enzymes, bacteria, algae, polysaccharides, fungi, DNA and human cell lines (Corclova and Ivanescu, 2018; Thliza *et al*., 2025). Synthesis of nanoparticles from plants and their extracts is advantageous due to their wide availability, safety in handling and possession of a wide variety of metabolites which may help in the reducing process, where plant extracts act as both reducing and stabilising agents of the metal nanoparticles. This is done through combining biomolecules such as proteins, amino acids and enzymes, polysaccharides, alkanoids, tannins, phenols, saponins, terpenoids and vitamins, which guarantee the stability of the nanoparticle (Frieger *et al*., 2021). Ovais *et al*. (2016) show plant extracts offer distinct advantages, especially for the synthesis of AgNPs, offering a cost-effective and efficient approach for the rapid synthesis of highly stable AgNPs, with the added advantage of shorter incubation times compared to other green synthesis methods, such as those involving fungi and bacteria.

Sustainable development goal no. 3, 'Ensure healthy lives and promote well-being for all ages', intends to end epidemics of AIDS, tuberculosis, malaria and other communicable diseases, thus achieving universal health coverage and providing access to safe and affordable medicines and vaccines for all.

Although antibiotics have been proved very effective in the fight against infectious diseases, inappropriate use and abuse have given rise to antibiotic-resistant pathogenic microbial strains (Osman *et al*., 2024; Selvaraj *et al*., 2024). This has necessitated the search for alternative plant-based medicines with antimicrobial activity. Therefore, the study on any nanoparticle with antimicrobial activity is an important addition to the tools for combating the menace, hence the need for this study on *Ficus thoningii* silver nanoparticles. This study aims to synthesise and characterise silver nanoparticles from the root extract of *Ficus thonningii* and evaluate its

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antimicrobial activities on selected pathogenic bacteria and fungi.

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MATERIALS AND METHODS

Reagents

All reagents used for the preparation of the silver nanoparticle were of analytical grade, purchased from **Sigma-Aldrich** with purity ranging from 95% to 98.8%, and used without further purification.

Roots of *the Ficus thonningii* plant were obtained from Lafia modern market in Nasarawa State, North Central Nigeria. They were pulverised to powder using a mortar and pestle and sieved using a 2 mm sieve, then stored in a polythene bag and kept for synthesis of the silver nanoparticle.

(A) Root of *F. thoningii* plant

(B) Powder from root of *F. thoningii*

The synthesis and characterisation of the nanoparticles was carried out at the Multiuser Research Laboratory, Ahmadu Bello University, Zaria. 10g of pulverised root powder of *Ficus thonningii* was transferred into an Erlenmeyer flask, and 52ml of methanol was added. The mixture was shaken on an ultrasonicator for about 30 minutes. The extract was filtered, and the residue was disposed of. A stock solution of 10 mM for $AgNO_3$ was prepared, from which 1-5 mM solutions were obtained. (Swamy *et al*., 2015)

10 ml, 20 ml, 30 ml, 40 ml, and 50 ml of 1 mM, 2 mM, 3 mM, 4 mM, and 5 mM silver nitrate solutions, respectively, were added to five separate 20 ml of the sample extract for the synthesis at a regulated temperature of 25° using an ultrasonicator and allowed to settle. The solution was filtered using Whatman No.1 filter paper to obtain a clear extract and stored in a refrigerator for characterisation and microbial activities investigation (Melkamu and Bitew, 2021).

1 cm^3 aliquots of the extract were diluted with 3 cm^3 of distilled water, a portion was transferred to a plastic cell of 1 cm path length, and UV-visible spectroscopy was run on an Agilent Cary 300 Spectrophotometer within the range of 350 nm to 800 nm according to the method of Veeraswamy and Ankur (2020). The formation of the silver nanoparticles was monitored at all concentrations investigated to confirm the reduction of Ag^+ to Ag^o between 0 minutes and 150 minutes at an interval of 30 minutes, monitoring the colour change. The FTIR spectrum of the synthesised nanoparticle was investigated using Agilent Cary 630 to identify functional groups present in the different samples. A sample of synthesised nanoparticles was placed on a prepared disc, compressed on the transparent disc, and a spectrum was obtained showing transmittance against the wave number of different functional groups. Values obtained were compared with those in the literature (Vahabi and Sedigheh, 2017).

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Scanning electron microscopy (SEM) was used to study the shape, particle size and the lattice image of the biosynthesised silver nanoparticle by placing a drop of the silver nanoparticle powder on a carbon-coated grid.

Antimicrobial Activity Studies

0.02 mg of the extract was weighed and dissolved in 10 ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 200 µg/mol. The diffusion method was used for screening the extract. Microbes were cultured on Mueller Hinton agar. The medium was prepared according to the manufacturer's instructions and sterilisedfor 15 minutes. It was poured into sterile petri dishes and allowed to cool and set. The prepared solution was seeded with 0.1 ml of the standard inoculum of the test microbes. The inoculum was spread evenly over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6 nm in diameter, a well was cut at the centre of each inoculated media. 0.1 ml of the solution of the extract of concentration of 200 µg/mol was then introduced into the well on the inoculated medium. Incubation was made at 3% for 24 hours, after which the plates of the medium were observed. The zone of inhibition of growth was measured with a transparent ruler, and the result recorded in millimetres.

Minimum Inhibitory Concentration

The minimum inhibition concentration of the extract was determined using the broth method. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilisedfor 15 minutes. The broth was allowed to cool. MC-Farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared, 10 ml was dispensed into a sterile test tube, and the test microbe was inoculated and incubated for 6 hours. Dilution of the test microbe was done in the normal saline until the turbidity matched that of McFarland's scale by visual comparison at this point. The test microbe has a concentration of about 1.5108 Cfu/ml. The fold serial solution of the extract was made in the sterile broth to obtain the concentrations of 200 µg/mol, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml. Having obtained the different concentrations of the extract in the sterile broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was done at for 24 hours, and thereafter observed for turbidity (growth). The antimicrobial investigation was carried out at the Bafawak diagnostics laboratory in Lafia, Nasarawa State, Nigeria.

RESULTS

Formation of Silver Nanoparticles

At 0 minutes, a sharp absorption change was observed on the absorption spectrum, indicating that the formation of silver nanoparticles started immediately after the two solutions were mixed. After 1 hour 30 minutes, repeated peaks were observed, suggesting complete formation. After another 24 hours, the same peaks were observed for all five different concentrations. The change in colour from reddish brown to a relatively clear brown solution indicated the reduction of silver from the (+1) to the (0) oxidation state (Plate 1).

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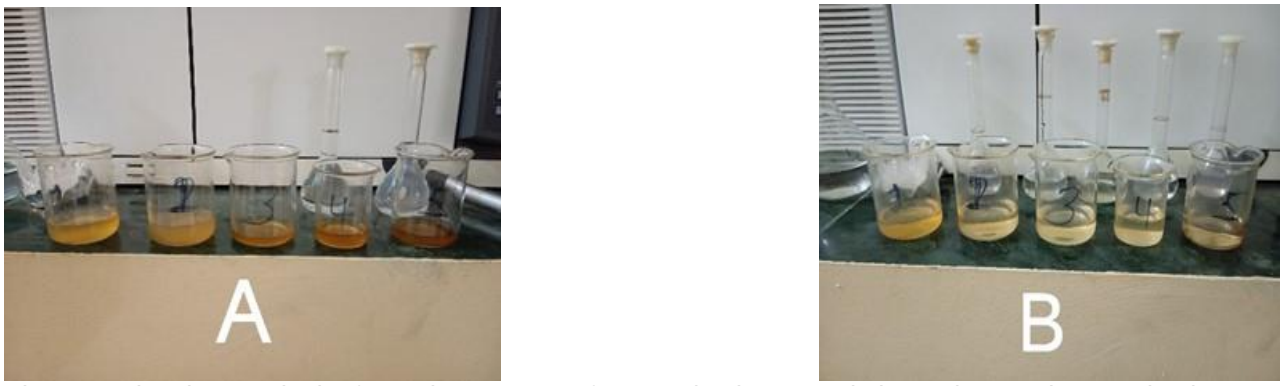

Plate 1. Color changes in the formation process of AgNPs in plate A and plate B in complete synthesis stage

Characterisation of AgNPs

UV-Visible Spectroscopy

The result of the UV-vis spectroscopy showed absorption between 350 nm and 800 nm, with a peak at 400 nm. Increasing silver nitrate intensity with time showed that more silver nanoparticles are produced in solution. The absorption spectra showing the maximum absorption peak of the AgNPs is shown in Figure 1.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the complex identified functional groups in the region of 4000 cm^{-1} - 400 cm^{-1} characteristic of flavonoids, alkaloids, saponins and other phenolic compounds associated with the AgNPs.

Formation of nanoparticles in 1mM, 2mM, 3mM, 4mM and 5mM solutions of silver nitrate was further confirmed from their Fourier transform infrared spectrum, as the silver-oxygen bond, which normally occurs at a range of 350 cm^{-1} - 500 cm^{-1}, was absent. The FTIR spectrum of the silver nanoparticles showed some notable peaks linked to the surface of silver nanoparticles responsible for the reduction. The identified functional groups are esters, alkenes, ketones, amides, hydroxyl, carboxylic and cyanide, with peaks shown in Figure 2 and functional groups in Table 1.

Table 1: Identified functional groups

Functional Groups/ Wavenumber Range (cm^{-1})	**Ethers** 1200-1070	**Alkenes** 1670-1610	**Ketones** 1800-1600	**CH_2** 1475-1400	**CH** 3000-2850	**NH** 3550-3000	**OH** 3600-3200	**COOH** 2500-3300	**CN** 2322-2138
1Mm	1028	1606	1982	1371	2855	3332	3332	2922	2098
2mM	1028	1602	1848	1438	2847	3339	3339	2914	2109
3mM	1028	1602	1714	1438	2855	3321	3321	2922	2124
4mM	1028	1602	1707	1438	2851	3287	3287	2914	2109
5mM	1028	1606	1915	1438	2851	3500	3649	2922	2322

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Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy showed surface morphology of the nanoparticles to be cubic and spiky in shape (Plate 2).

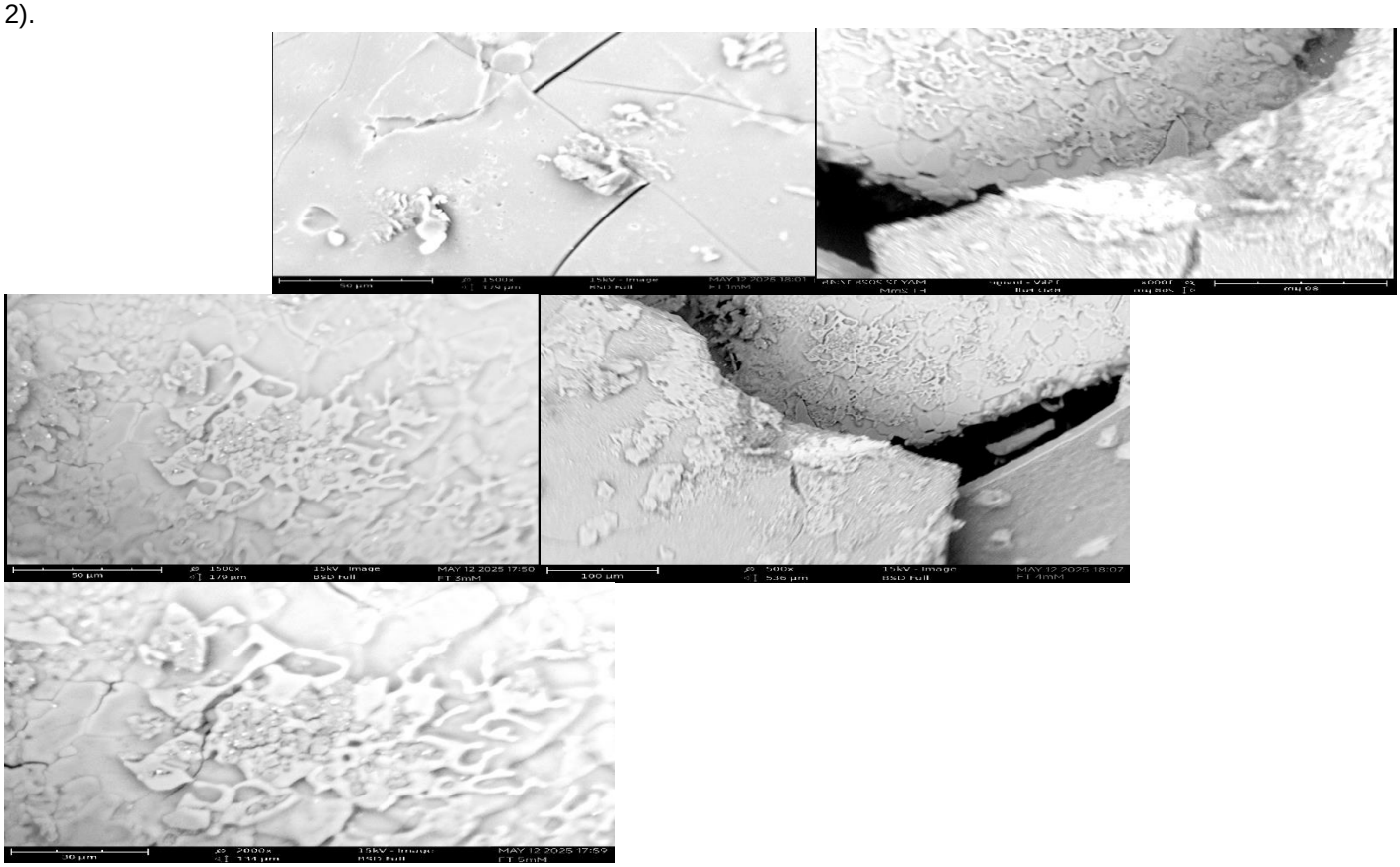

Plate 2. SEM Images of 1mM to 5mM AgNPs synthesized from *Ficus thoningii*\

Antimicrobial Studies

The antimicrobial activities (sensitivities) results (Table 2) showed varying degrees of sensitivity of the organisms to the nanoparticles and standard antibiotics. All four bacteria species were sensitive to 4 mM. *S. aureus* and *E. coli* were highly sensitive, *S. typhi* very sensitive and *C. jejuni* sensitive. Four organisms were also sensitive to the silver nanoparticles at 2 mM; *S. aureus*, E. coli and *C. jejuni* are very sensitive, while S. typhi is sensitive. At 5 mM, *S. aureus* and *C. jejuni* were very sensitive, while *S. typhi* was sensitive. The three fungal pathogens tested showed no sensitivity. In comparison, all four organisms were sensitive to Gentamicin and Ciprofloxacin. Three organisms were sensitive to streptomycin. C. albicans and A. flavus were sensitive to ketoconazole, while *A. flavus* and *F. oxysporium* were sensitive to fluconazole. The minimum inhibitory concentration for all bacteria studied was 3 mg/mL, and the bactericidal effects range from 1.33 to 1.70 (Table 5). At the tested range of concentrations, there was no inhibition on fungal growth.

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Table 2: Antimicrobial Activities (sensitivity) of Synthesized AgNPs on Pathogenic Bacteria and Fungi and selected Antibiotics.

Concentration(mM)	*S.aureus*	*E.coli*	*S.typhi*	*C.jejuni*	*C.albicans*	*A.flavus*	*F. oxysporium*
1	-	-	-	-	-	-	-
2	++	++	+	++	-	-	-
3	-	-	-	-	-	-	-
4	+++	+++	++	+	-	-	-
5	++	-	+	++	-	-	-
Gentamycin	++	++	+++	++	-	-	-
Ciprofloxacin	+++	+++	++	++	-	-	-
Streptomycin	-	+++	+++	++	-	-	-
Augmentin	-	-	-	-	-	-	-
Ketoconazole	-				+	+++	-
Fluconazole	-	-	-	-	-	+++	+

Key

(-) = resistant

(+) = sensitive

(++) = very sensitive

(+++) = highly sensitive

Table 3. Zone of Inhibition for the AgNPs Complex of selected Bacteria and Fungi

Concentration(mM)	*S.aureus*	*E.coli*	*S.typhi*	*C.jejumi*	*C.albicans*	*A.flavus*	*F.oxysporium*
1	6	5	4	4	0-1	0	0-1
2	10	8	9	9	0-2	0-1	0-2
3	14	12	11	12	1-3	0-2	1-3
4	18	15	13	14	1-4	1-2	1-3
5	20	16	14	15	2-5	1-3	1-4

Table 4: Bactericidal Effects of the AgNPs on pathogenic Bacteria.

Organisms	*MIC*	*MBC*	*MBC/MIC*	*Effects*
S.aureus	3	5	1.70	Bactericidal
E.coli	3	4	1.33	Bactericidal
S.typhi	3	4	1.33	Bactericidal
C.jejumi	3	5	1.70	Bactericidal

Key

MBC - lowest concentration that produced no growth on subculture from inhibited wells (i.e., bactericidal concentration).

MIC - lowest concentration with no visible growth in broth (or very clear inhibition on plate).

Table 5. Fungicidal Effects of the AgNPs on pathogenic Fungi.

Organisms	*MIC*	*MFC*	*MFC/MIC*	*Effects*
C.albicans	-	-	-	No Activity
A.flavus	-	-	-	No Activity
F.oxysporium	-	-	-	No Activity

Key

MIC - lowest concentration with no visible growth in broth (or very clear inhibition on plate).

MFC - lowest concentration that produced no growth on subculture from inhibited wells (i.e., fungicidal concentration).

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DISCUSSION

Colour change was the indicator that nanoparticles were synthesised from $AgNO_3$ and Ficus thonningii root extract. The colour change confirmed the reduction of silver ions (Ag+) to silver atoms (Ag0). This has been the basis for confirmation of the formation of AgNPs in *Prunus persica*, *Acacia cyanophylla, Thevetia peruviana* and *Azadirachta indica*. (Panigrahi, 2013; Oluwaniyi *et al.*, 2015; Kumar et al., 2017; Jalab *et al*., 2021; Thliza et al., 2025).

The colour of the solution changed from light yellow to brown and eventually turned dark brown when silver salt ($AgNO_3$) was added to the aqueous pine needle extract. This colour change is attributed to the presence of various phytochemical compounds such as alkaloids, flavonoids, saponins, and steroids in the plant extract, which act as reducing agents responsible for the reduction of silver ions to silver nanoparticles. Similar colour changes have been observed by many researchers using different plant extracts [31–33]. The UV-visible spectra absorption peak of around 400 nm observed for Ficus AgNPs is consistent with AgNPs UV peak ranges of 350 nm to 500 nm *(Sharma et al., 2009; Cohen et al., 2020*) and similar to findings on nanoparticles of other trees such as *Acacia concinna* (430 nm), *A. leucophloea* (433 nm), *Prunus persica* (366.2 nm) and *A. cynophylla* (460 nm) *(Bilal et al., 2017; Alaalah, 2020; Thliza et al., 2025).*

FTIR spectra identified functional groups such as alcohols, phenols and carboxylic acids, which are known to be responsible for the reduction and stabilisation of formed AgNPs. The variety of functional groups present in nanoparticles are known to act as reducing, capping and stabilising agents in metal nanoparticle synthesis. The various functional groups can have different roles. The –OH can donate electrons to metal ions, reducing them to their elemental forms, acting as both reducing and stabilising agents; –COOH can participate in the reduction process to enhance the solubility; $–NH_2$ can facilitate electron transfer during the reduction of metal ions to make them effective reducing agents; and C=O can facilitate complex formation with metal ions essential for controlling NPs' size and morphology during synthesis. This helped to confirm the synthesis of the silver nanoparticles. The SEM gave the shape of the Figus AgNPs to be cubic and spiky. The shape is mostly attributed to the extract type used in the synthesis (Flieger et al., 2021). The Ficus AgNPs demonstration of highly sensitive antimicrobial activity against all four tested bacteria, *C. jejuni, E. coli, S. typhi and S. aureus, shows* high efficacy of this synthesised complex as an antibacterial. The absence of Ficus AgNPs activity against the fungal species studied, *A. flavus, C. albicans and F. oxysporium,* suggests that the complex either has no antifungal activity or does so only at concentrations higher than those in the study.

The bioactivity of AgNPs is well documented, and the effect of the silver ion components and the phytochemical compounds, such as terpenoids, saponins, flavonoids and other phenolic compounds, are both known to be bioactive by themselves and have now been shown to produce stronger pathogen elimination than either alone. This demonstrates the general principle that metallic nanohybrids combined with bioactive partners produce enhanced antimicrobial potency, otherwise known as the synergistic effect (Musimun et al., 2022; Wronska et al., 2023). This makes these plant-extract-based or biogenic nanoparticles a vital addition to the antibacterial arsenal and to the other functions they perform.

CONCLUSION

Ficus thoningii root extract successfully formed silver nanoparticles with $AgNO_3$ at a peak of 400 nm. Functional groups found to be associated with the complex are typical of terpenoids, flavonoids, saponins and other phenolic compounds, and the shape of the complex was found to be cubic and spiky.

Bacteria *(C. jejuni, E. coli, S. typhi and S. aureus)* were highly susceptible to the complex, making it a very important addition to the antibacterial arsenal and other functions they perform.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research.

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APENDICES

PLATES AND FIGURES

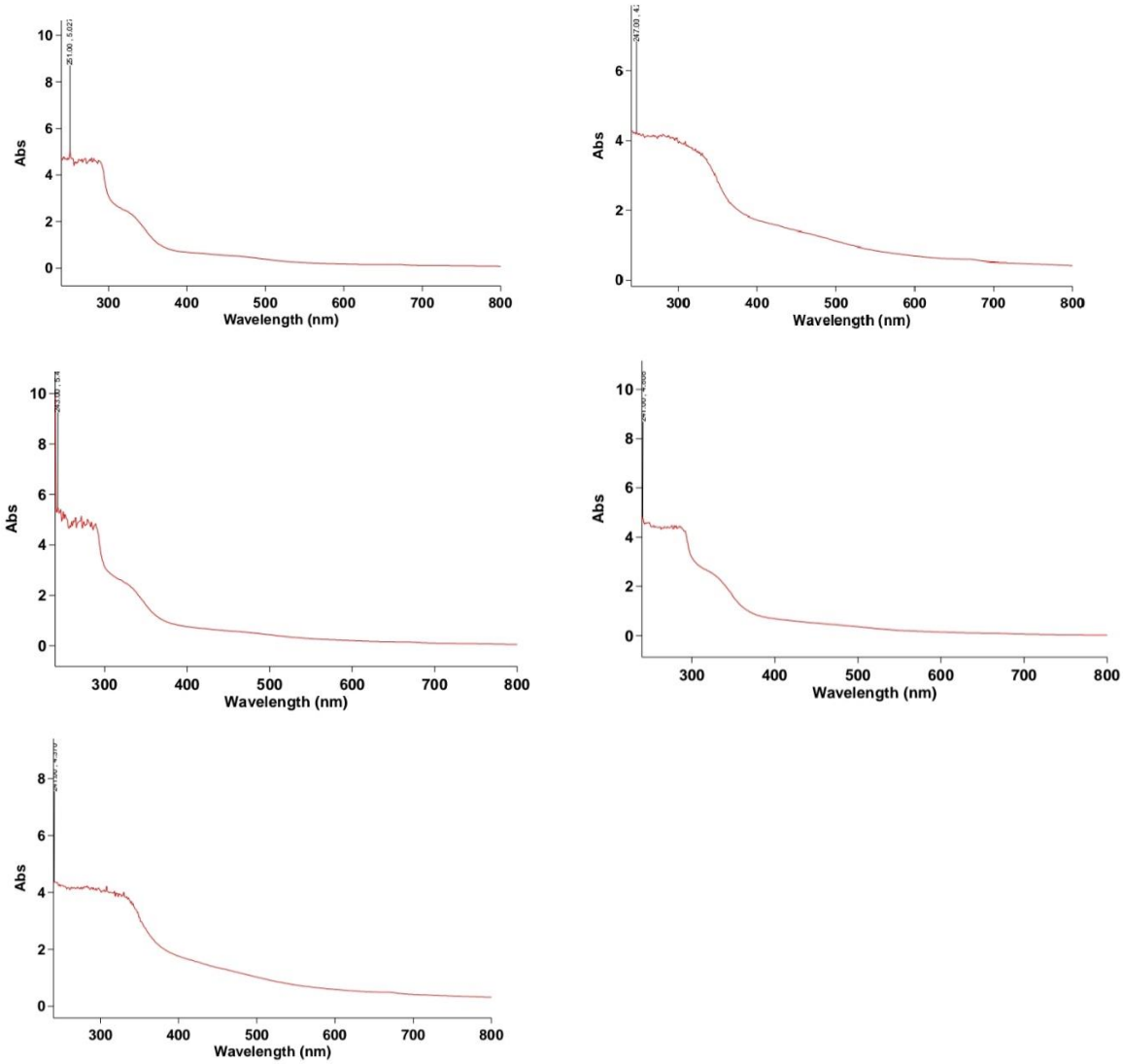

Figure 1: UV-Visible Spectra of the synthesized silver nanoparticles

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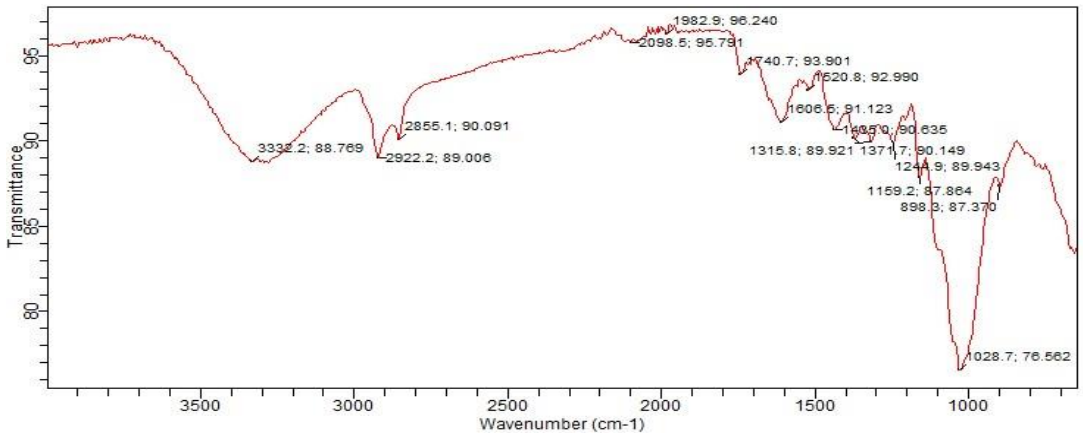

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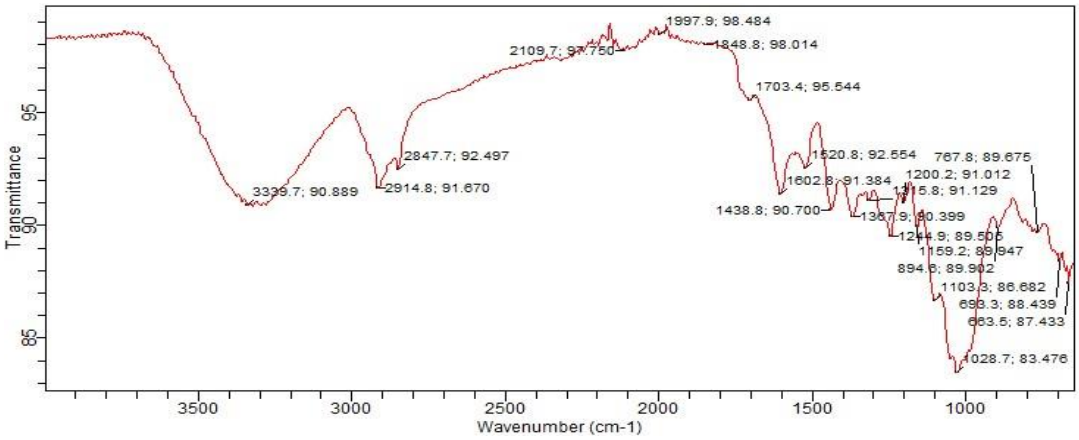

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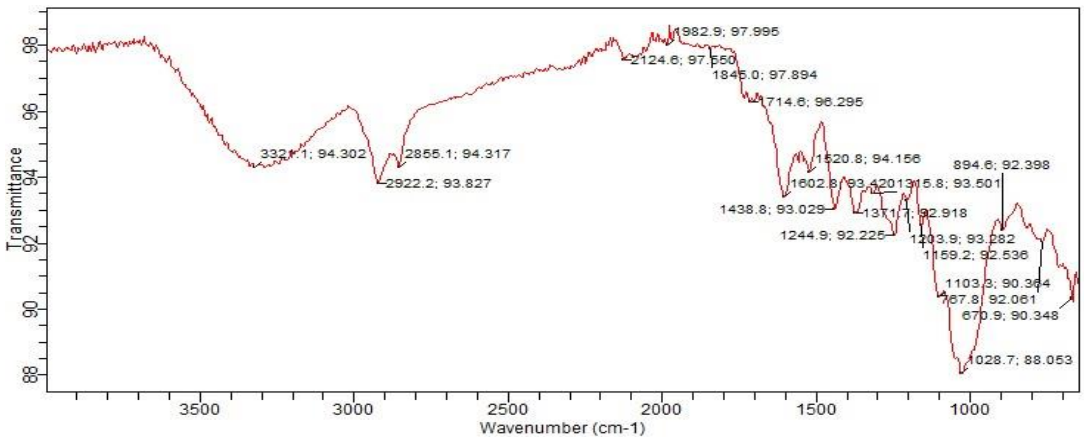

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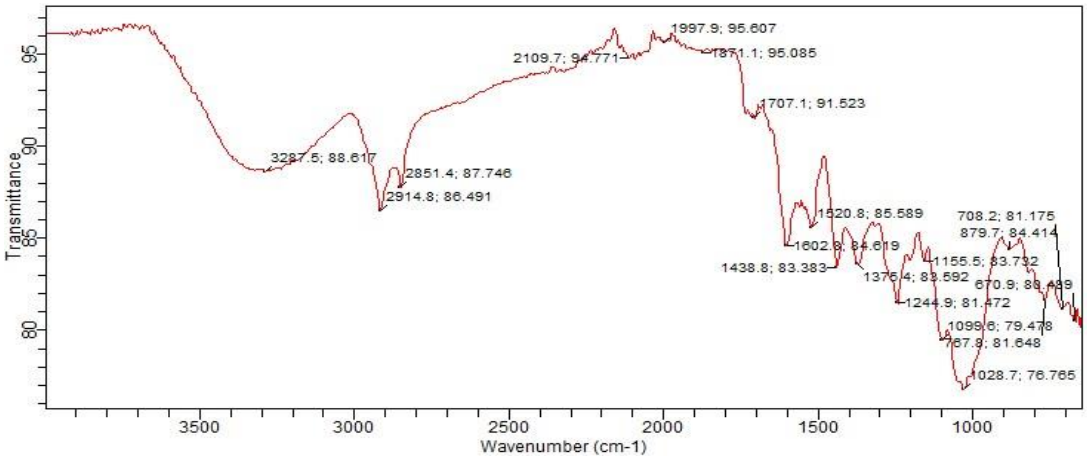

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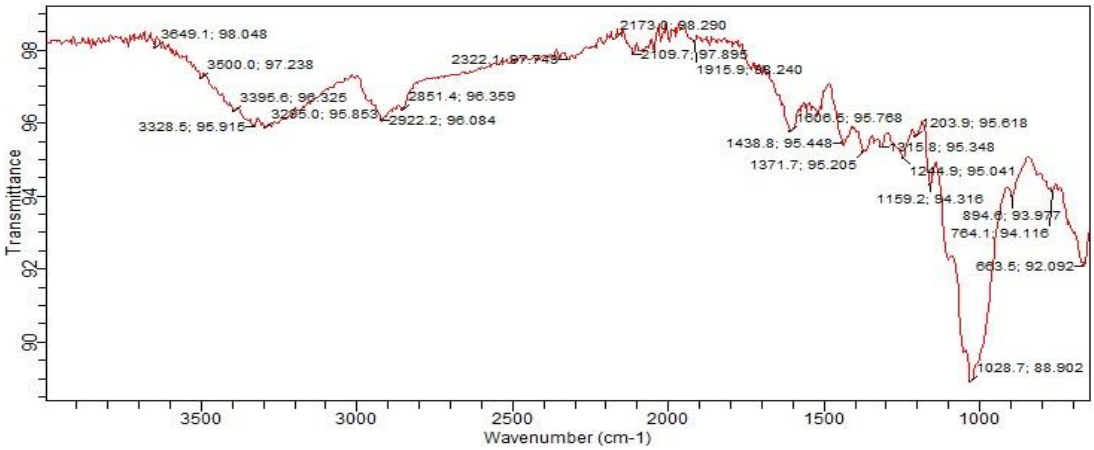

e.

Figure 2. FTIR Spectra of the synthesized silver nanoparticles at concentrations 1-5mM (a-e)