



Research Article

Comparative antibacterial activity of green-synthesized silver nanoparticles and solvent fruit extracts of *Acacia nilotica* (L.) Willd. against *Staphylococcus aureus* and *Salmonella Typhi*

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Abstract

Background: Green-synthesized silver nanoparticles (AgNPs) are utilized to treat antimicrobial-resistant bacteria. This study investigated the antibacterial activity of ethyl acetate, methanolic, and hexane extracts of *Acacia nilotica* fruits, as well as biosynthesized silver nanoparticles, against *Staphylococcus aureus* and *Salmonella typhi*.

Methods: *Acacia nilotica* fruit powders were extracted using methanol, hexane, and ethyl acetate at concentrations of 25%, 50%, and 75% weight per volume. Silver nanoparticles were synthesized from the aqueous extract of fruits and silver nitrate, and the disc diffusion method was performed.

Results: Change of solution color, ultraviolet-visible spectroscopy (UV-Vis), and Fourier transform infrared spectroscopy (FTIR) confirmed the successful synthesis of silver nanoparticles (The UV-Vis spectrum showed a surface plasmon resonance (SPR) peak at ~445 nm). FTIR results indicated the presence of biomolecules involved in silver (Ag⁺) reduction, including nitro compounds, amides, carboxyl groups, alcohols, benzene, and phenols. Hexane extract with a concentration of 75% inhibited *S. typhi* (19.67 ± 2.081) and *S. aureus* (11.33 ± 2.081), whereas Hexane extract with a 25% concentration had no effect against *S. aureus*. Ethyl acetate extract with 75% concentration inhibited *S. aureus* (16.33 ± 0.577), while ethyl acetate 75% and 50% and methanol 25% and 75% had no effect on *S. typhi*. Methanol 75% inhibited *S. typhi* (13 ± 0.1). AgNPs 25% and 50% inhibited *S. aureus* (17 ± 1.0 and 15 ± 1.0 , respectively) and exhibited low activity against *S. typhi*. Control showed no inhibition zones.

Conclusion: AgNPs of *A. nilotica* fruit inhibited *S. aureus* more than *S. typhi*; for *S. typhi*, standard antibiotic discs produced measurable inhibition zones. Hexane showed greater efficacy against *S. typhi* than AgNPs. Further investigations are needed to identify specific antimicrobial components.

Keywords: silver nanoparticles, *Acacia nilotica*, *Staphylococcus aureus*, *Salmonella typhi*, antibacterial activity, disc diffusion method, inhibition zones

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1. Introduction

Medicinal herbs have historically been used for combating diseases. They have been widely used in all countries for healthcare, given their biological properties, accessibility, and safety [1]. Besides the increasing rates of bacterial, fungal, and viral infections, there has been an increase in antibiotic resistance and side effects, which has decreased the safety and effectiveness of common antibacterial drugs [2].

Today, creating nanoparticles using herbs and their products is crucial in nanotechnology, medical products, pharmaceuticals, and biotechnology research. This approach is also more environmentally friendly, easier to synthesize, and more cost-effective [3]. The synthesis of metal nanoparticles is a key area of study in materials research, with applications in magnetic, optoelectronic information storage, electronics, and drug delivery systems [4]. Silver nanoparticles are used in nanobiotechnology studies. Due to their catalytic activity and antimicrobial, anticancer, and bactericidal effects, they can act as agents to fight wound infections. Compared to other metals, silver exhibits strong antibacterial activity, high toxicity to microorganisms, and lower toxicity to mammalian cells [5, 6]. The synthesized silver nanoparticles (AgNPs) from actinomycetes show significant potential as anticancer and antibacterial agents [7].

Acacia nilotica belongs to the family *Fabaceae*. The fruits (pods) of this plant are greenish black and indehiscent, with a necklace-like appearance [8]. Traditionally, the plant has been used as an anti-cancer, diuretic, and astringent agent, aiding in the treatment of intestinal pain, colds, diarrhea, cough, congestion, fever, dysentery,

leucorrhoea, gallbladder issues, hemorrhages, ophthalmic issues, sclerosis, smallpox, and tuberculosis [9].

The methanolic (fruits) extract of *A. nilotica* exhibited both Gram-positive and Gram-negative antibacterial activities [10], along with mild antibacterial activity against multidrug-resistant *Salmonella typhi*. AgNPs derived from *A. nilotica* demonstrated broad-spectrum antibacterial effects at lower concentrations [11]. AgNPs made from *A. nilotica* methanolic bark extract showed good antibacterial efficacy against both Gram-positive and Gram-negative bacteria [12]. Biosynthesized AgNPs from *A. nilotica* extract possess antibacterial properties, indicating potential for medical applications and holding promise for further use in healthcare settings [13], especially given their anticancer, anti-diabetic, and antioxidant activities [14].

Staphylococcus aureus (golden staph) is a round-shaped, Gram-positive bacterium that causes skin infections, such as abscesses, respiratory infections, and food poisoning [15]. *Salmonella typhi* is a Gram-negative *bacillus* and is the leading cause of typhoid fever.

This work aims to synthesize silver nanoparticles from the aqueous fruit extract of the widely used Sudanese medicinal plant (Garad), also known as *A. nilotica* (L.) Willd, and to evaluate its antibacterial activities at three different concentrations against a Gram-positive and a Gram-negative bacterium. This study compares the activities of extracts prepared with three solvents (ethyl acetate, methanol, and hexane) at three different concentrations using the agar disc diffusion method.

2. Methods

2.1. Materials

2.1.1. Plant

A. nilotica (L.) Willd fruits were purchased from an herbalist at Omdurman market on February 26, 2017, and identified at the herbarium of the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan. The voucher specimen was numbered and preserved in the herbarium.

2.1.2. Bacteria

Staphylococcus aureus and *S. typhi* were used in this study. They were isolated in the microbiology laboratory of the Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, at the University of Khartoum. They were identified through cultural, morphological, and biochemical tests, including catalase, oxidase, coagulase, urease, mannitol fermentation, and DNA tests.

2.2. Methodology

2.2.1. Plant extracts preparation

For preparing the plant extract, 50 g of dried *A. nilotica* fruit powder was extracted using a Soxhlet apparatus with methanol, hexane, and ethyl acetate for 6 hrs. The extracts were filtered, evaporated, and diluted to 25%, 50%, and 75% weight per volume (w/v) concentrations with distilled water. The extracts were stored in a refrigerator for further studies.

2.2.2. Silver nanoparticles synthesis

- Aqueous plant extract preparation: To prepare the aqueous extract, 50 g of powder was soaked in 50 ml of distilled water overnight and then filtered [16].

- Preparation of silver nitrate solution and synthesis of AgNPs: To prepare a 1.0 mM solution of silver nitrate (AgNO_3), 8.49 mg of AgNO_3 were weighed and dissolved in 50 mL of distilled water. Silver nanoparticle biosynthesis was carried out by mixing 10 mL of aqueous plant extract with 5 mL of a 1.0 mM AgNO_3 solution. The mixtures were incubated in the dark at 30°C for 24 hours to prevent photochemical reactions. The synthesized AgNPs were then centrifuged twice at 10,000 rpm for 5 mins, collected, and stored for further studies [13].

2.2.3. Testing antibacterial activity

The extracts and the synthesized AgNPs were tested for antibacterial activity against *S. aureus* and *S. typhi*. Concentrations used were 25%, 50%, and 75% w/v. The culture was inoculated using a spread plate with broth culture suspension. The plates were then incubated at 37°C for 18–24 hours and observed [16]. The comparative antibacterial effectiveness of the extracts was assessed against the tested microorganisms using the disc diffusion method [17, 18]. Antibacterial activity was indicated by inhibition zones surrounding the discs containing the extracts. These zones were measured and expressed in millimeters [16]. Distilled water served as a negative control, while gentamycin and tetracycline were used as positive controls, with disc amounts of 10 μg and 30 μg , respectively. The activity index (AI) was calculated using the formula: Inhibition zone of the plant extract / Inhibition zone

of the antibiotic. Five replicates were performed for each assay.

2.2.4. Silver nanoparticles analysis by UV-visible spectrophotometer and FTIR

Ultraviolet-visible (UV-Vis) spectroscopic analysis was performed using a Shimadzu UV-1800. A cuvette with a 10 mm path length was used. Measurements were taken as a function of reaction time at room temperature.

Fourier Transform Infrared Spectroscopy (FTIR) analysis of dried AgNPs was conducted using pellets of potassium bromide (KBr; FTIR grade) in a 1:100 ratio. The spectrum was recorded with a Jasco FT/IR-8400 spectrometer. The FTIR spectrometer, equipped with a JASCO IRT-7000 Infrared Microscope and operating in transmittance mode at a resolution of 4 cm⁻¹ (JASCO, Tokyo, Japan), was used for measurements.

2.3. Statistical analysis

The variations in antibacterial activity among different treatments were evaluated by measuring inhibition zones and analyzed using ANOVA in SPSS 28, with significance set at $P < 0.05$.

3. Results

3.1. AgNPs formation

The synthesis of AgNPs using *A. nilotica*'s aqueous fruit extract and AgNO₃ was confirmed by the bio-reduction of silver ions, as indicated by a color change from pale yellow to dark brown (Figure 1).

3.2. Antibacterial activities of extracts

The antibacterial activities of methanol, hexane, and ethyl acetate extracts from the fruits of *A. nilotica* at concentrations of 25%, 50%, and 75% were tested against two bacteria: *S. aureus* and *S. typhi*. The results, showing the diameters of the zones of inhibition in mm, are presented in Table 1 and Figure 2. The hexane extract at 75% concentration demonstrated higher inhibition against *S. typhi* (19.67 ± 2.081) and *S. aureus* (11.33 ± 2.081), while 25% concentration had no effect on *S. aureus* (0 ± 0.0). The ethyl acetate extract at 75% inhibited *S. aureus* (16.33 ± 0.577), but ethyl acetate at 50% and 75%, as well as methanol at 25% and 75%, showed no effect on *S. typhi* (0 ± 0.0). Methanol extract at 75% inhibited *S. typhi* (13 ± 0.1). The activity index of *A. nilotica* extracts was generally low, except for the hexane extract at 75% against *S. typhi* (1.22).

There is no statistically significant difference in inhibition zones among different extract types (ethyl acetate, methanol, and hexane at various concentrations, $P = 0.659$). The bacterial types (*S. aureus* vs *S. typhi*) also show no significant difference (P -values > 0.05).

3.3. AgNPs antibacterial activities

The antibacterial activity of AgNPs was tested on bacterial colonies on nutrient agar plates, supplemented with AgNPs (Table 2; Figures 3 & 4). The inhibition zones observed indicated that the prepared AgNPs had antibacterial effects against *S. aureus* at 25% and 50% concentrations, measuring 17 ± 1.0 and 15 ± 1.0 mm, respectively. The activity against *S. typhi* was lower, showing an 8 mm zone at 25%. Organisms in water were used as control plates and showed no inhibition zones.

In this study, it was observed that the antibacterial activity of the synthesized AgNPs decreased as the concentration increased, while activity in solvent extracts increased with concentration.

AgNP concentration was nearing a significant effect on inhibition zones ($P = 0.050$). The type of bacteria has a highly significant effect, with *S. aureus* showing greater susceptibility than *S. typhi*.

3.4. Comparative analysis

As shown in Figure 5, *S. aureus* was generally more susceptible to both the extracts from solvents and AgNPs, especially those from methanol and ethyl acetate. Conversely, *S. typhi* was resistant to ethyl acetate and methanol but was more sensitive to hexane extracts and responded moderately to AgNPs. Hexane extracts were found to be more effective than AgNPs.

Statistically, silver nanoparticles were slightly more effective against *S. aureus*, but the difference

was not statistically significant. For *S. typhi*, both treatments demonstrated similar inhibition with no significant difference detected.

3.5. UV-Vis and FTIR spectroscopic studies

The absorption peak in UV-Vis spectroscopy was observed in the visible range at 445 nm. Due to the plasmon resonance effect, this peak indicated the successful formation of AgNPs. Different active biological compounds appeared in FTIR. The synthesized AgNPs with biomolecules contributed to the reduction of Ag^+ to Ag^0 by adhering to the surface of the AgNPs (Figure 6). The intense peaks at 1200 cm^{-1} , 1530 cm^{-1} , 1600 cm^{-1} , 1680 cm^{-1} , and 3415 cm^{-1} corresponded to the presence of C-O alcohols, N-O nitro compounds, C=O carboxylic acids, C-H aromatic groups, O-H of alcohols, and phenols, respectively.

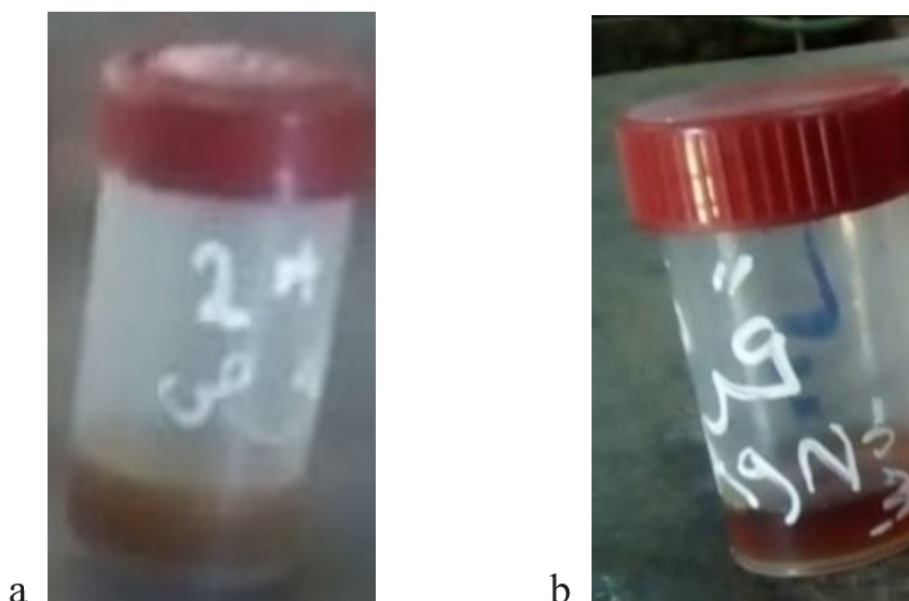
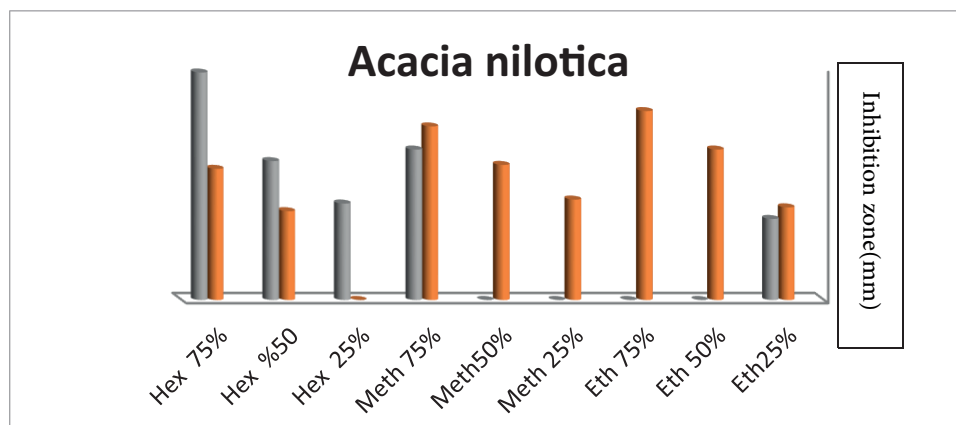


Figure 1: *A. nilotica* fruits. (a) Color of the aqueous extracts. (b) Color of the silver nanoparticle extracts.

Table 1: Antibacterial activities of extractives of *A. nilotica* against *S. aureus* and *S. typhi*.

Plant extract	<i>S. aureus</i>			<i>S. typhi</i>		
	IZ (mm)	AI _{gent.}	AI _{tetra}	IZ (mm)	AI _{gent.}	AI _{tetra}
Eth25%	8 ± 0.1	0.26	0.29	7 ± 0.1	0.44	0.29
Eth 50%	13 ± 0.1	0.42	0.46	0 ± 0.0	0	0
Eth 75%	16.33 ± 0.577	0.53	0.58	0 ± 0.0	0	0
Meth 25%	8.67 ± 1.527	0.28	0.31	0 ± 0.0	0	0
Meth 50%	11.67 ± 1.527	0.38	0.42	0 ± 0.0	0	0
Meth 75%	15 ± 0.1	0.48	0.54	13 ± 0.1	0.81	0.54
Hex 25%	0 ± 0.0	0	0	8.33 ± 1.537	0.52	0.30
Hex 50%	7.67 ± 0.577	0.25	0.27	12 ± 1.0	0.38	0.35
Hex 75%	11.33 ± 2.081	0.37	0.40	19.67 ± 2.081	1.22	0.69
Gentamicin	31 ± 1.0			16 ± 1.0		
Tetracycline	28 ± 1.0			24 ± 1.0		

Eth, ethyl acetate; Meth, methanol; Hex, hexane; IZ, inhibition zone; AI, activity index; Gent., gentamicin; Tetra, tetracycline.

**Figure 2:** Antibacterial activities of extracts of *A. nilotica* against *S. aureus* (red) and *S. typhi* (green).**Table 2:** Inhibition zones of the aqueous AgNP concentrations against the tested bacteria.

Plant silver nanoparticle concentration	<i>Staph aureus</i>			<i>Salmonella typhi</i>		
	IZ (mm)	AI _{gent.}	AI _{tetra.}	IZ (mm)	AI _{gent.}	AI _{tetra.}
<i>A. nilotica</i> 25% AgNo ₃	17 ± 1.0	0.55	0.61	8 ± 1.0	0.50	0.33
<i>A. nilotica</i> 50%Ag No ₃	15 ± 1.0	0.48	0.54	7 ± 1.0	0.44	0.29
<i>A. nilotica</i> 75% AgNo ₃	14 ± 1.0	0.45	0.50	6 ± 1.0	0.38	0.25
Gentamicin	31 ± 1.0			16 ± 1.0		
Tetracyclin	28 ± 1.0			24 ± 1.0		

IZ, inhibition zone; AI, activity index; Gent., gentamicin; Tetra., tetracycline.

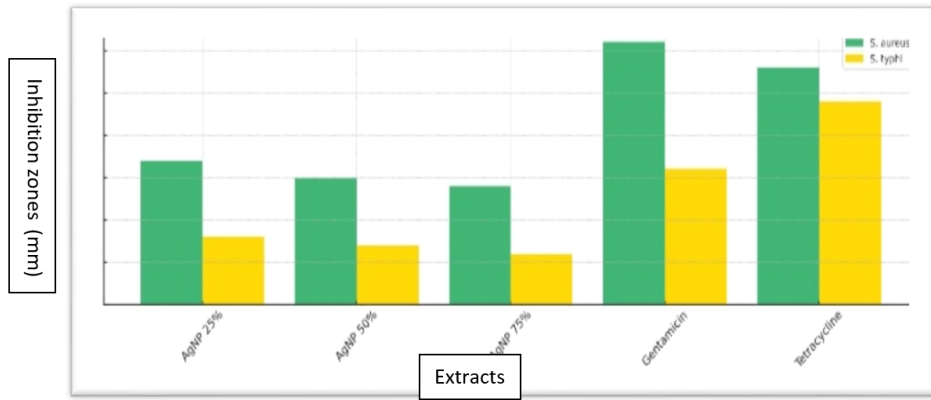


Figure 3: Inhibition zones of the aqueous AgNP concentrations against the tested bacteria.

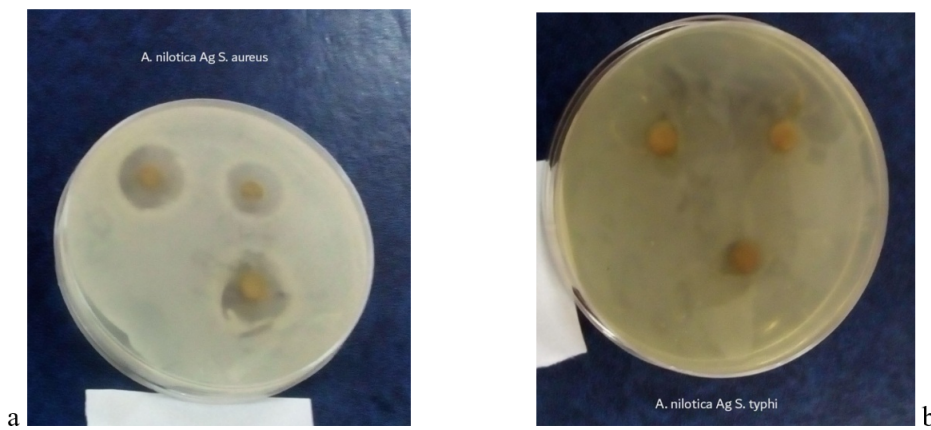


Figure 4: Inhibition zones of the aqueous AgNP of *A. nilotica* concentrations 25%, 50%, and 75% against: (a) *S. aureus* and (b) *S. typhi*.

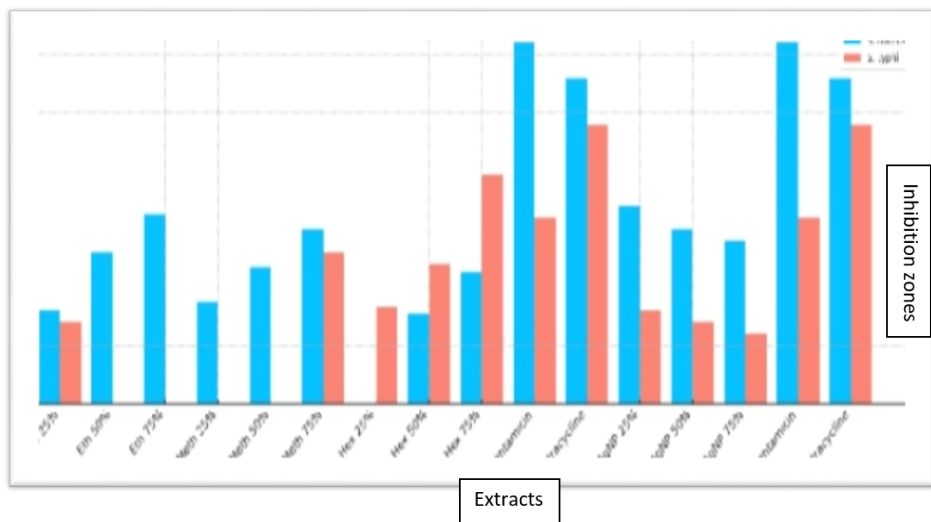


Figure 5: Comparison of antibacterial activity of crude extracts and AgNPs from *A. nilotica*. *Staphylococcus aureus* is shown in blue and *S. typhi* in red.

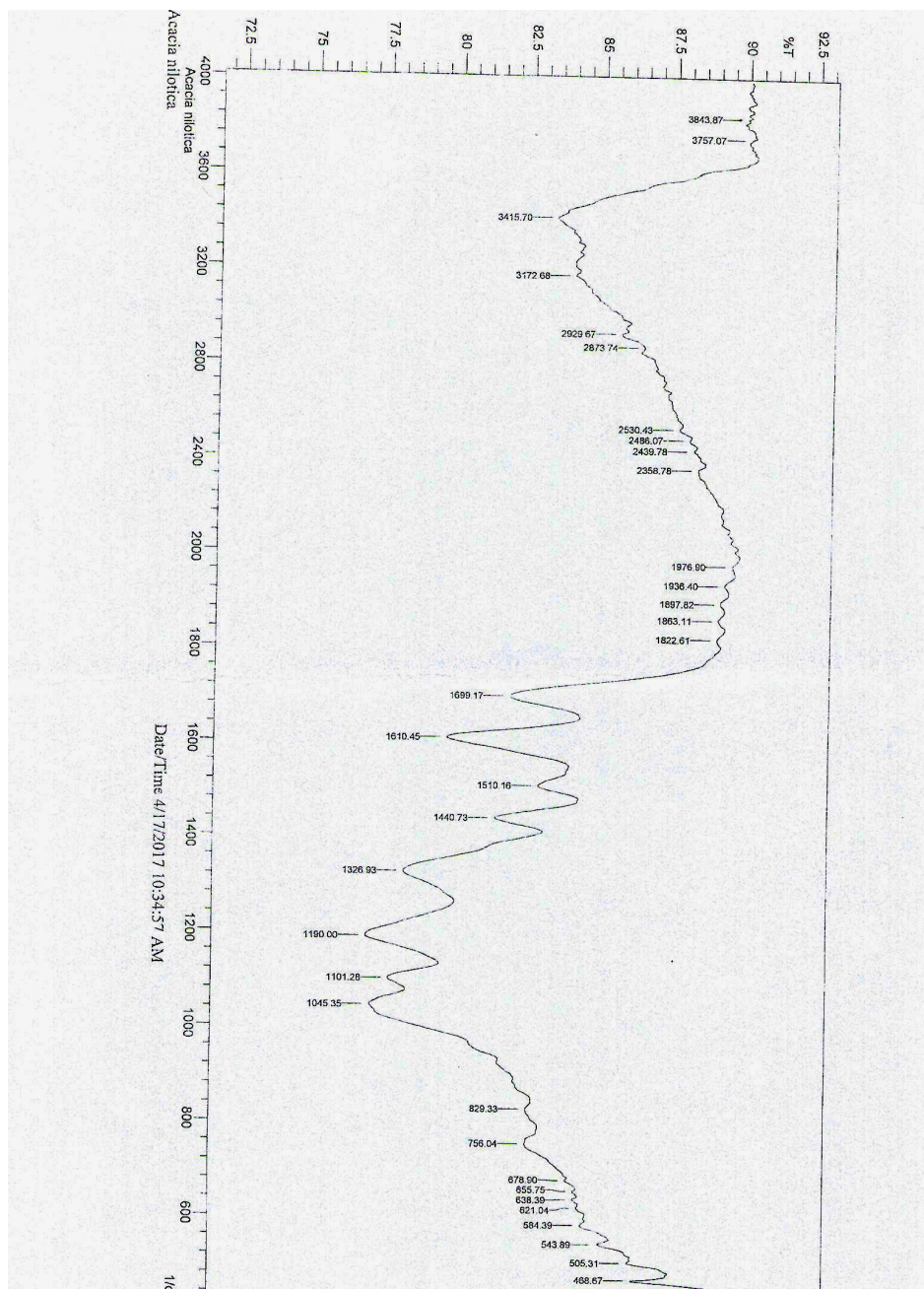


Figure 6: FTIR of *A. nilotica*.

4. Discussion

AgNPs were found to be more effective against *S. aureus* because Gram-positive and Gram-negative bacteria differ in cell wall structure and composition. This finding aligned with previous studies. AgNP of *A. nilotica* was reported to possess higher antibacterial activity and larger inhibition zones against Gram-negative bacteria,

and the largest inhibitory zones against Gram-positive ones [14]. In our study, methanolic extracts showed limited activity against *S. typhi*. Methanol at 25% and 75% had no effect on *S. typhi* compared to previous studies; *A. nilotica* (fruits) methanolic extract formed the largest inhibition zone against the Gram-negative *S. typhi* at a 100 mg/mL concentration [19]. This difference may be due to the varying concentrations used. Other studies

have stated that *Acacia* extracts exhibit significant antibacterial activity against antibiotic-resistant bacteria [20]. In this study, the maximum response of the hexane extract was against *S. typhi*, more so than the synthesized AgNPs, although it was less effective against *S. aureus*. Hexane is non-polar, and some non-polar compounds have been shown to disrupt bacterial cell membranes, especially against Gram-negative bacteria like *S. typhi*, which possess lipopolysaccharides and can be penetrated more effectively by lipophilic (non-polar) substances compared to polar compounds extracted by methanol or ethyl acetate.

Results obtained in previous studies [21, 22] advocated the antibacterial activity of AgNPs against resistant bacteria in medicine. Synthesized AgNPs from plants can kill bacterial cells. Because of their interaction with DNA, enzymes, and proteins, they cause physical and chemical changes in bacterial cell membranes.

5. Conclusion

This study concludes that noble metals, such as silver, can be utilized efficiently and cost-effectively. Metallic nanoparticles, combined with plant metabolites, showed *in vitro* activity and may help combat multidrug resistance; clinical translation is not inferred. Changes in solution color, UV-Vis, and FTIR spectroscopy results indicated successful synthesis of AgNPs. Extracts from *A. nilotica* fruits showed greater effects against *S. aureus* than against *S. typhi*. AgNPs made from *A. nilotica* fruit aqueous extract demonstrated high activity against *S. aureus* and low activity against *S. typhi*. *Salmonella typhi* is more resistant and shows low susceptibility. The maximum response of the hexane extract was observed against *S. typhi*.

5.1. Study Limitations and Recommendations

The extracts of this plant need further analysis to identify the specific antimicrobial compounds responsible for its activity against other bacteria and fungi. Additional research on the AgNPs of this plant should include advanced nanoparticle characterization techniques (TEM/SEM, XRD, DLS), assessment of extraction yield, and quantification of total phenolics and flavonoids.

Declarations

Acknowledgments

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Ethical Considerations

All work was conducted following proper safety measures and standard operating procedures to ensure researcher and environmental safety. No human or animal subjects were involved in this study.

Competing Interests

None.

Availability of Data and Materials

Data are available upon reasonable request to the corresponding author.

Funding

None.

Abbreviations and Symbols

AgNPs: Silver nanoparticles

UV-Vis: Ultraviolet-visible spectroscopy

FTIR: Fourier transform infrared spectroscopy

KBr: Potassium bromide

AI Use Disclosure

The authors declare that no generative AI tools were used in the preparation of this manuscript.

Author Contributions

Both authors are equally accountable for all aspects of the work.

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