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Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Leaves Extract of *Ficus sycomorus* (Sycamore Fig) and its Antimicrobial Evaluation

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Abstract
An eco-friendly zinc oxide nanoparticle production using F. sycomorus leaves extract as reducing and stabilizing agents was accomplished. The green-produced ZnO NPs were examined using UV-vis, SEM, FTIR, and XRD. *The presence of a distinctive SPR peak at 320 nm induced by* harmonic oscillation of electrons in the conduction band of the spectrum *revealed the production of ZnO NPs by the UV-Vis. The functional groups incorporated in the produced ZnO NPs were detected by FTIR analysis. The generated zinc oxide nanoparticles have developed a spherical hexagonal wurtzite structure, according to the SEM examinations. Using Debe Sherer's equation, the XRD measurements showed that the nanosized ZnO had a hexagonal morphology with an average size of 18.94 nm. Amoxicillin and fluconazole were used as antibiotic and antifungal controls at a concentration of 10 µg/mL each. The ZnO NPs at varying concentrations of 12.50, 25.0, and 50.0(mg/mL) were evaluated against each pathogen demonstrated strong antibacterial action against the minimum inhibition zones of the Gram-positive bacteria S. aureus (21 mm) and S. typhi (18 mm), respectively. However, it displayed no activity for Klebsiella spp. and were active against Gram-negative bacteria E. coli (24 mm) minimum inhibition zone. The antifungal activity shows the highest activity with the synthesized ZnO NPs against Candida albicans (28 mm). The findings of this work indicate that F. sycomorus leaf extract has bio-reducing properties for the production of ZnO nanoparticles, and the resultant nanoparticles can be utilized efficiently as effective antimicrobial agents for biological applications.*

Keywords: Green synthesis, Zinc Oxide, Nanoparticles, *Fiscus sycomorus*, Antimicrobial

Introduction
Nanotechnology is considered one of the most advanced technologies of the twenty-first century, with the goal of developing novel materials at the nanoscale (Patel *et al*., 2021), in this multidisciplinary field, matter with sizes ranging from 1 to 100 nm is created, manipulated and can be applied in many areas (Dhaka *et al*., 2023; 2024). Nanoparticles and nanomaterials are increasingly being used in innovative ways because of their completely new or enhanced size, distribution, and shape-based features. This has resulted in the creation of materials with unexpected macro tunnel effects, surface area, volume, and quantum size. Nanoparticles (NPs) are incredibly small particles that, because of their incredibly small surface area to volume ratio, have enhanced thermal conductivity, chemical stability, non-linear optical performance, and catalytic reactivity (de Jesus *et al*., 2024). Nanotechnology is a crucial component of numerous significant technologies through nanoscale structures in the domains of optics, electronics, biomedical science, mechanics, drug-gene delivery, chemical industry,

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optoelectronic devices, nonlinear optical devices, catalysis, space industries, energy science, and photoelectrochemical applications (Singh *et al*., 2019; Raj *et al*., 2021). There are two main methods for creating nanoparticles: conventional and green synthesis methods. Green synthesis involves using microorganisms such as bacteria, fungi, and algae, as well as natural materials like leaves, roots, and flower extracts, and enzymes that allow large-scale production of NPs free of additional impurities (Salem *et al*., 2021). According to *Hosseingholian et al*. (2023), the green synthesis method is a desirable substitute for conventional methods because it is environmentally sustainable, eco-friendly, non-toxic, biodegradable, and inexpensive. In contrast, conventional methods of producing ZnO NPs are linked to high costs, environmental pollution, substantial energy consumption, and other issues (*Dogaroglu et al*., 2024). Metal oxides, including zinc, gold, copper, silver, nickel, and others, are becoming more and more important; recent studies show the need for producing environmentally sustainable metal oxide nanoparticles (Kumar *et al*., 2021). According to Bala *et al*. (2015), zinc oxide nanoparticles are semiconductors that crystallize in either a hexagonal wurtzite or a cubic zinc mix structure. They are frequently referred to as II-VI binary compounds. According to Galdamez-Martinez *et al*. (2020), ZnO NPs exhibit a wide band gap, excellent electron mobility, good transparency, and strong room-temperature luminescence, among other special qualities. Zinc oxide nanoparticles have garnered attention recently because of their numerous uses in biomedical systems, electronics, and optics (Jamdagni *et al*., 2016). ZnO NPs produced through green synthesis exhibit excellent antibacterial properties, surpassing chemically synthesized versions, while being non-toxic and safe for various applications (Dogaroglu *et al.,* 2024).

The current work focuses on using aqueous leaf extracts to create environmentally compatible ZnO nanoparticles from the *Ficus sycomorus* plant, a semi-deciduous tree belonging to the family *Moraceae* that can grow up to 20-21 m tall, maximum 46 m (Erhirhie *et al*., 2018). It grows well in areas with well-drained, loamy, clay, and sandy soils (Kassa *et al.,* 2015). In addition to treating jaundice, the leaves are utilized as an antidote for snakebite (Nuhu *et al*., 2024). Elkobrosy *et al.,* (2023) cited that *Ficus sycomorus* has been shown to have characteristics that prevent the growth of bacteria, and the plant's aqueous extract contains a number of active phytochemical ingredients. Its common names include bush fig, fig-mulberry, sycamore fig, strangler fig, and Baure in Hausa, Tarmur in Kanuri, Asoro in Yoruba, Ukwa in Igbo, and Ibbi in Fulfulde.

(**a**) tree (**b**) leaves

Figure 1: ***Ficus sycomorus*** **Plant**

MATERIALS AND METHODS

Materials

The materials used during this research work include *Ficus sycomorus* leaves, distilled water, zinc acetate dihydrate solution ($Zn(C_2H_3O_2)_2.2H_2O$) Sigma Aldrich, sodium hydroxide solution (NaOH), DMSO, (Sigma Aldrich) peptone water, nutrient agar, incubator, centrifuge machine, drying oven, blast furnace, weighing balance, and thermometer, among others. Every chemical used in this study was of analytical quality.

Methods

Plant collection

Fresh leaves of *Ficus sycomorus* were collected in the Katanga vicinity, which is about 12 kilometers northeast of Federal University Dutse, Jigawa State, and were authenticated by Dr. Mohammed Isah Auyo, a taxonomist at the herbarium section of the Department of Biological Sciences, Federal University Dutse, Jigawa State. Taxonomic code: FUDHAN: 004/43/2.

Plant Extract preparation

Fresh leaves of *Ficus sycomorus* were properly cleaned with flowing tap water and then rinsed with distilled water. The healthy leaves were air-dried for about five days at the chemistry laboratory, Federal University Dutse, Jigawa State. After being dried, the leaves were crushed with a mortar and pestle into a fine powder and sieved. About 5 g of finely ground *Ficus sycomorus* powder were taken and mixed with 100 mL of distilled water in an Erlenmeyer conical flask, which was then heated for half an hour at 50–60 degrees Celsius. The extract was then filtered using Whatman Filter Paper No. 1 after being allowed to cool to room temperature. The resulting extract was kept for further examination at 4 °C. This is in accordance with the reports of (Naiel *et al.,* 2022; Ramanarayanan *et al.,* 2018) with little modification.

Green synthesis of Zinc oxide Nano particles

About 50 mL of *Fiscus sycomorus* leaf extract solution was combined with approximately 100 mL of a produced 0.1 M zinc acetate dihydrate solution (Zn(C2H3O2)2.2H2O) in an Erlenmeyer conical flask while being continuously stirred. The mixture was vigorously stirred for one hour at 60 °C with a magnetic stirrer. The colour changed from reddish-brown to pale yellow, which was considered a visual indicator for the formation of zinc oxide NPs. After washing, the crude was centrifuged for ten minutes at 4500 rpm, and the supernatant was disposed. Eventually, after ten hours of drying at 40°C in an oven, the pellets were crushed into a fine powder with a mortar and pestle, and then placed in airtight vials for additional examination. With minor adjustments, this is consistent with the findings of (Kandwal *et al*., 2019; Jayachandran *et al*., 2021).

Characterization techniques

UV–Vis spectral analysis

An environmentally friendly reduction of Zn (II) ions to zinc oxide NPs by *F. sycomorus* leaves extract has been examined at the Biochemistry Laboratory, Federal University, Dutse, Jigawa State, using a UV-visible spectrophotometer (Analytikjena) in the 200–800 nm range.

FTIR analysis

FTIR spectroscopy analysis was used to identify the functional groups that were present in the biosynthesized ZnO NPs. Using the KBr pellet method, FTIR analysis was carried out in

Perkin-Elmer Spectrum at the central research laboratory of Umaru Musa Yar'adua University, Katsina State.

XRD analysis

Using a PAN analytical diffractometer (model: AR'XTRA X-ray, serial number 197492086), the synthesized ZnO nanoparticles' XRD pattern shows the crystalline structure of the produced ZnO NPs in the 2θ range.

SEM examination

The scanning electron microscope machine at Umaru Musa Yar'adua University's central research laboratory in Katsina State, model number PRO X: 800-07334 Phenom World, serial number MVE01570775, was used to measure the shape and size of the generated ZnO nanoparticles.

RESULTS AND DISCUSSION

UV-visible spectroscopy

Zinc (II) ions undergo reduction to zinc oxide in the solution by secondary metabolites derived from *F. sycomorus* leaves. This was established by examining the UV-visible spectrum between 200 and 800 nm, as shown in Fig 2. At 320 nm, the spectra displayed a prominent absorbance peak that is unique to ZnO nanoparticles. According to reports, the absorbance peak for ZnO nanoparticles occurs between 310 and 360 nm in wavelength. This result aligns with previously reported literature. (Jayachandran *et al.*, 2021).

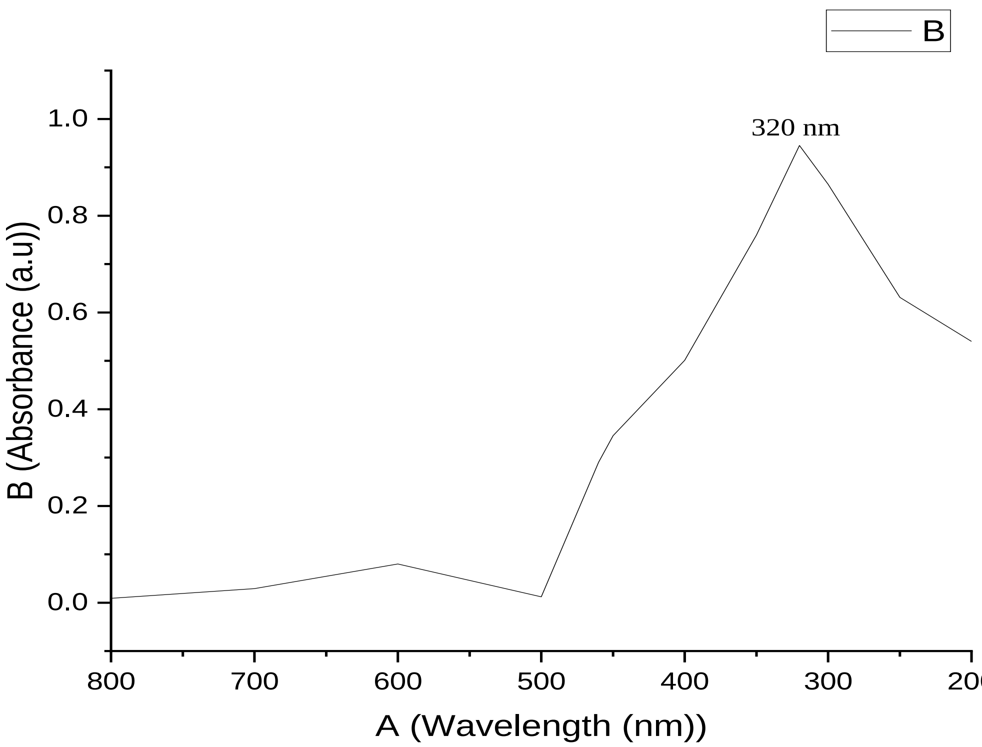

Figure 2: Ultraviolet-Visible spectrum of ZnO nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR)

The Fig 3 displays the spectrum that was obtained after the functional groups in the biosynthesized ZnO NPs were identified using FTIR spectroscopy. It was found that the broad absorption band at 3369.51 cm^{-1} was caused by the O-H group of plant phenolics or alcohols. The peak at 2684.90 cm^{-1} was used to identify the alkane (C-H) bond in stretching mode. The amine (N-H) group of proteins or enzymes found in the leaf extract of *F. sycomorus* is represented by the peaks at 1564.52 and 1380.98 (cm^{-1}). The absorption peaks found at 654.81 cm^{-1} confirmed that Zn-O bonds were present in the generated NPs. The peak at 871.87 cm^{-1} represents the Zn-O bond's stretching frequency. A peak at 2100.77 cm^{-1} indicates that an aliphatic or aromatic amine is in the C–N stretching phase. The bio-reduction of Zn^{2+} ions to ZnO NPs was assisted by the phenolic chemicals or proteins present in the leaf extract of *F. sycomorus*, as demonstrated by the appearance of N-H and O-H bonds in the FTIR spectrum. These findings are consistent with earlier research (Muthukumaran *et al*., 2018), with slight modifications.

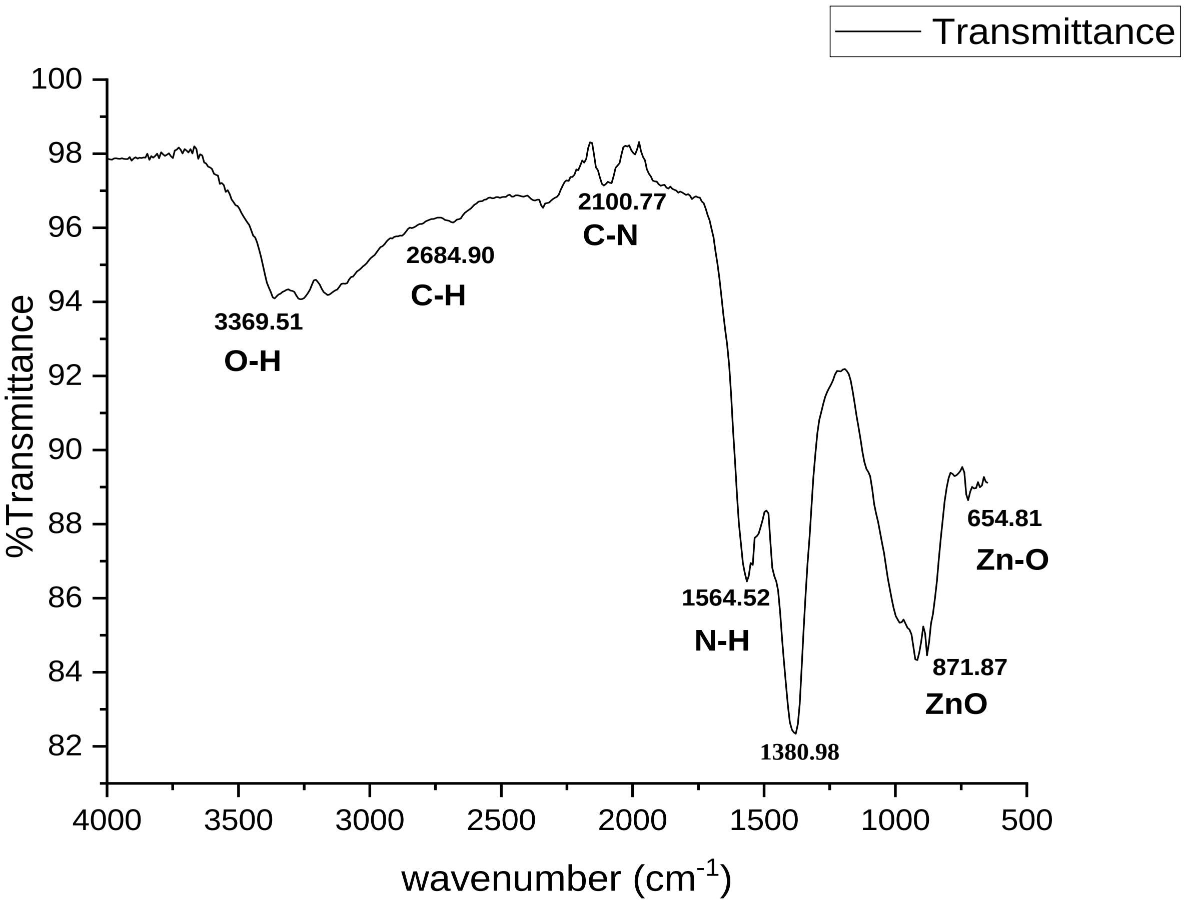

Figure 3: Synthesized Zinc Oxide NPs' FTIR spectrum

Scanning Electron Microscopy (SEM)

The size and shape of the produced ZnO nanoparticles at various magnifications were determined by SEM investigations. SEM images of the produced ZnO, which were spherical hexagonal wurtzite particles, were shown in Fig 4. This was further confirmed by X-ray diffraction (XRD) investigation. ZnO NPs' dimensions and morphologies have considerable influence on the effectiveness against microbial pathogens. ZnO NPs with hexagonal nanoparticles are more effective against numerous kinds of microorganisms (Andia-Huaracha *et al*., 2022). These results correspond with previous studies (Rusli, 2024).

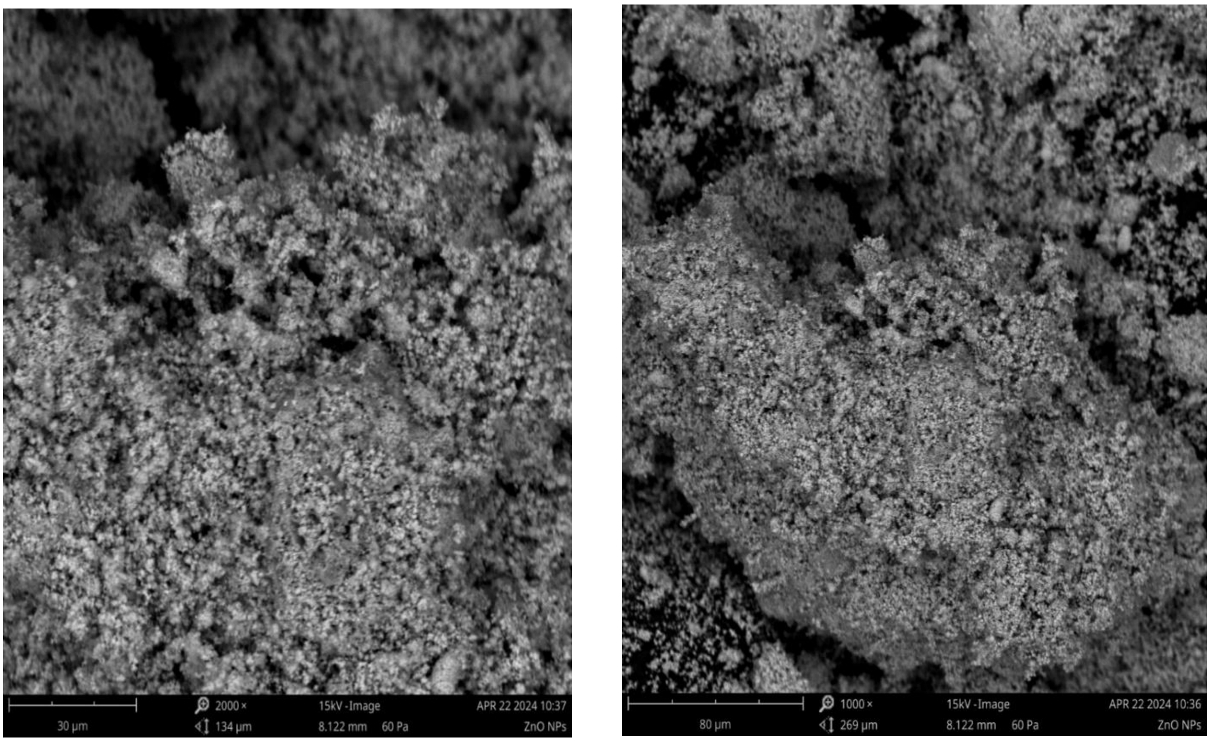

Figure 4: SEM images for the synthesized ZnO NPs

Diffraction of X-Rays (XRD)

The Fig 5 displays the XRD pattern of the biosynthesized ZnO nanoparticles. The product has a pure and crystalline structure, as indicated by the sample's diffraction pattern matching the hexagonal wurtzite ZnO standard X-ray diffraction peaks JCPDS 00-36-1451. The reflections denoted by the XRD peaks at 31.86, 34.50, 36.33, 47.55, 56.55, 62.81, and 67.97 theta values are (100), (002), (101), (102), (110), (103), and (112), respectively. Similar XRD findings for Zn nanoparticles were also published in the literature. (Aminuzzaman *et al.,* 2018). According to Scherrer's equation, the average crystal size of the generated ZnO NPs was 19 nm as shown in Table 1..

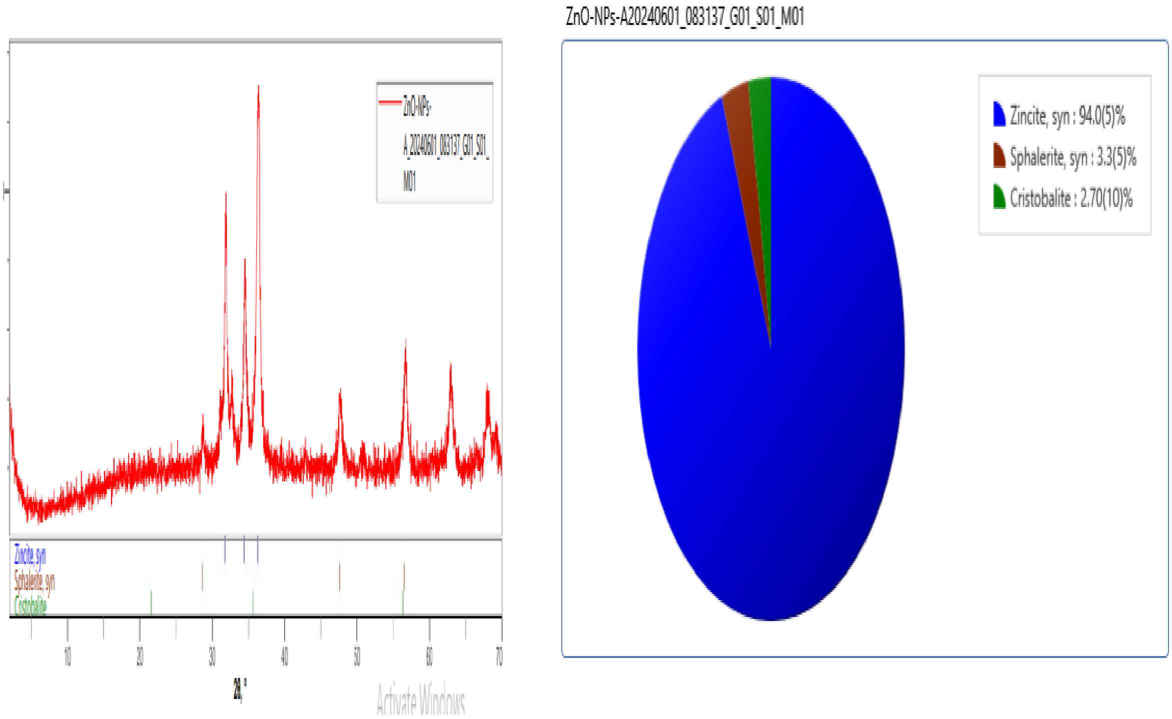

Figure 5: The generated XRD Pattern of ZnO NPs'

Table 1: XRD Lattice Characteristics of ZnO NPs'.

Peak location (2 theta)	FWHM	Crystallite Size D (nm)	D (nm) Average
28.73	0.31	26.45382018	19
31.857	0.4	20.65372395	
32.73	0.39	21.23008166	
34.5	0.35	23.76701004	
36.33	0.459	18.21563798	
47.55	0.51	17.02156079	
56.554	0.58	15.55303059	
62.811	0.66	14.10285944	
67.97	0.71	13.49415842	

Antimicrobial Activity of Synthesized ZnO Nps

The produced ZnO NPs were tested for antimicrobial properties against clinically isolated G^{+ve} bacteria (*S. aureus, S. typhi, and Kleb. spp*.), G-ve bacteria (*E. coli*), and *Candida albicans* fungal isolates, as shown in Table 2. Nutrient agar medium (NA) was prepared for 15 minutes at 121°C, then transferred into petri dishes and kept at 30°C for 24 hours. Subsequently, NA plates were coated with the bacterial solution and infected. The slider was then used to gently brush the plate's surface. Additionally, three paper discs containing the synthesized ZnO NPs in varying concentrations of 12.5, 25.0, and 50 (mg/mL) were made using DMSO. These discs were then put on the agar plates along with the antibiotic control amoxicillin (AM) and kept at 37°C for a whole day. The zone of inhibition's diameter was measured using a scale with little modification, the result is consistent with the report of (Akbar *et al.*, 2019; Aliyu *et al.*, 2024).

Table 2. Antimicrobial evaluation of the synthesized ZnO NP

Isolate	Zone of Inhibition (mm)			Positive control	
	12.5mg/mL	25mg/mL	50mg/Ml	GEN	FLU
S. aureus	15	18	21	14	-
S. typhi	12	16	18	13	-
E. coli	16	22	24	11	-
Klebb. Spp	0.0	0.0	0.0	0.0	-
C. albican	23	25	28	-	12

Key: GEN: Gentamicin, 10µg FLU: Fluconazole, 10µg

Conclusion

The results of this work show that the aqueous leaf extract of *Fiscus sycomorus* is a useful resource for the environmentally friendly generation of ZnO NPs. The different characterization results validate the successful production of ZnO NPs with the desired properties for antibacterial applications. The findings from the antimicrobial assessments suggest that the synthesized ZnO NPs have the potential to be efficient antimicrobial agents against a range of pathogens.

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