ANTIFUNGAL, ANTIMICROBIAL AND LARVICIDAL ACTIVITIES OF ZINC OXIDE NANOPARTICLES SYNTHESIZED FROM THE LEAF EXTRACT OF *BALANITES AEGYPTIACA*

Abstract

This study investigates the Antifungal, Antimicrobial and Larvicidal Activities Zinc oxide nanoparticles (ZnO-NPs) produced from the leaf extract of *Balanites aegyptiaca*. The nanoparticles were characterized using UV-Visible spectrophotometry, FTIR, XRD, and SEM techniques. Their antimicrobial activity was assessed against two Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), two Gram-negative bacteria (*Salmonella typhi, Klebsiella pneumoniae*), and two fungi (*Candida albicans, Aspergillus niger*). The ZnO-NPs demonstrated strong inhibitory effects on *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Salmonella typhi*. Larvicidal activity against *Anopheles* mosquito larvae at concentrations of 40, 50, and 60 mg/L showed 100% mortality in first instar larvae, with LC_{50} values ranging from 36.24 to 38.07 mg/L across instars. These results highlight the strong dose-dependent larvicidal and antimicrobial properties of ZnO-NPs derived from *Balanites aegyptiaca* leaf extract demonstrating their potential as eco-friendly nanobiopesticides and plant-based therapeutics for controlling disease vectors and pathogens.

Keywords: Anopheles Larvae, Antimicrobial, *Balanites aegyptiaca* (Leaf), Green Synthesis, Larvicidal Activity, Nanoparticles,

INTRODUCTION

Nanoparticles are materials with at least one dimension smaller than 100 nanometers, possessing distinctive characteristics such as a high surface area-to-volume ratio, specific crystalline structures, adjustable pore sizes, and the capacity to affect cellular and molecular processes in living systems [1]. Generally ranging from 1 to 100 nanometers, these particles are engineered or modified at the atomic scale. Their nanoscale size imparts physical and chemical properties that are markedly different from those of bulk materials, with the increased surface area further enhancing their reactivity and functionality [2].

Nanoparticles (NPs) encompass the characterization, design, and fabrication of biological and non-biological structures measuring less than 100 nanometers, distinguished by their unique and novel functional properties [3]. Interest in NPs has grown rapidly, driven by their superior electrochemical reactivity, thermal conductivity, and nonlinear optical behavior, which open avenues for diverse applications [4]. In particular, metal nanoparticles have attracted substantial attention owing to their exceptional features, including high surface area, stability, ease of chemical modification, suitability as fillers to enhance permeability, and flexible synthesis

methods. Nanoparticles can be synthesized in sizes ranging from the micrometer to the nanometer scale through either top-down (destructive) or bottom-up (constructive) methods. Zinc oxide (ZnO) is recognized as a biologically safe material with notable photo-oxidizing and photocatalytic effects on chemical and biological systems [2]. In its nano-sized form, ZnO can adopt various morphologies and has been widely reported to exhibit strong antibacterial activity against diverse bacterial species [5]. Both micro- and nano-scale ZnO are being investigated for antibacterial applications; however, reducing ZnO to the nanometer scale significantly enhances its antimicrobial potential. This is due to the nanoparticles' increased surface interaction with bacterial membranes and their ability to potentially penetrate bacterial cells. Such interactions trigger distinctive bactericidal mechanisms, with the primary mode of action involving toxic interactions that compromise bacterial cell integrity. These properties make nano-sized ZnO particularly valuable for antimicrobial applications, including use in the food industry [6]. Zinc oxide nanoparticles (ZnO-NPs) possess strong antibacterial properties, largely attributed to their high specific surface area resulting from reduced particle size, which enhances surface reactivity [2]. They have garnered considerable global research interest for their broad-spectrum biological activity, relative non-toxicity to human cells, biodegradability, and potential to improve the bioactivity of pharmacophores. Compared to bulk ZnO, ZnO-NPs display superior antibacterial efficiency due to quantum confinement and other size-dependent effects. Their ability to inhibit bacterial growth through multiple pathways makes them highly effective against bacterial contamination-related diseases—an increasingly critical advantage as conventional antibiotics face diminishing efficacy due to rising bacterial resistance [7]. The biosynthesis of zinc oxide nanoparticles (ZnO-NPs) using extracts from medicinal plants, fungi, bacteria, and algae enhances their stability and biocompatibility in diverse biological environments. This green synthesis approach also modifies their physicochemical properties, thereby improving their biological performance. Additionally, ZnO-NPs serve as efficient nanocarriers for conventional drugs due to their affordability, biodegradability, and compatibility with living systems [8]. Their versatility has led to applications in areas such as drug delivery, biosensing, gene therapy, nanomedicine, bioimaging, coatings for medical implants, electronic sensors, wastewater treatment, and communication technologies [9].

Balanites aegyptiaca (L.) Del., commonly referred to as the "desert date," is an evergreen, woody, spiny flowering tree that can reach heights of up to 10 meters. Belonging to the Balanitaceae family, it is widely distributed across arid regions of Africa and southern Asia. The plant is a rich source of bioactive compounds, including saponins, flavonoids, alkaloids, lipids, proteins, carbohydrates, and organic acids. Traditionally, various parts of *B. aegyptiaca* have been used in herbal medicine to treat numerous ailments [10]. It is recognized for its diverse pharmacological properties, including antidiabetic, anthelmintic, antibacterial, and antiviral activities. Additionally, the bark, unripe fruits, and leaves have been reported to possess anthelmintic, antifertility, purgative, and antidysentery effects [11].

Balanites aegyptiaca is a widely cultivated desert plant valued for its diverse applications, particularly in arid and semi-arid regions of Africa, the Middle East, and South Asia [12]. Traditionally, various parts of the plant have been used in the treatment of ailments such as skin boils, leukoderma, malaria, wounds, colds, syphilis, and disorders of the liver and spleen. The

fruit, known as the desert date, is the most prominent part of the tree. This drupe is initially pubescent when unripe, turning yellowish and smooth upon ripening, and consists of four distinct layers: the outer skin (epicarp), fleshy pulp (mesocarp), woody shell (endocarp), and the inner seed (kernel). All four layers have potential applications in both industrial and pharmaceutical fields. The edible components—particularly the pulp and kernel—are sources of oil, while the pulp is nutritionally rich in carbohydrates (62.63%) and protein (9.19%), with lower levels of fat (2.58%) and dietary fiber (2.93%) [13]. The fruits are commonly used by local communities to treat various health conditions [14]. Specifically, the fruit is employed in the treatment of jaundice, and oil extracted from the seeds serves as a laxative and is traditionally used to manage hemorrhoids, stomach ailments, jaundice, yellow fever, syphilis, and epilepsy [11].

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of *Balanites aegyptiaca* were collected from the field within the premises of the University of Maiduguri, Borno State, Nigeria, and taken to the Department of Plant Science for proper identification and authentication. Sampling was conducted during the dry season, after which the leaves were sorted and stored in clean polythene bags for subsequent analysis.

Anopheles mosquito larvae were obtained from various identified breeding sites within Gombe metropolis using a ladle and collection bottles. For sampling, the ladle was positioned at an angle of about 45° and gently lowered into the water until one side was just beneath the surface, minimizing disturbance to prevent the larvae from diving. The collected larvae were then transferred to the laboratory, where they were maintained and reared for larvicidal bioassay experiments. Collection procedures followed the method described by Abba *et al*. [9].

Sample Preparation

Leaves of *Balanites aegyptiaca* were collected, thoroughly rinsed with distilled water, and air-dried in the shade to minimize phytochemical degradation. The dried leaves were then ground into a fine powder using a mechanical blender.

Preparation of *Balanites aegyptiaca* Leaf Extract

The aqueous extraction of *Balanites aegyptiaca* leaves followed the method described by Al-Senani (2020) [15], with slight modifications. Ten grams (10 g) of powdered sample were mixed with 200 mL of distilled water in a 250 mL conical flask and heated in a water bath at 60 °C for 3 hours with continuous stirring. The mixture was then allowed to cool to room temperature and filtered through Whatman No. 1 filter paper. The freshly prepared aqueous extract was subsequently used for the synthesis of zinc oxide nanoparticles.

Synthesis of Zinc Oxide Nanoparticles.

Twenty millilitres (20 mL) of *Balanites aegyptiaca* leaf extract were combined with 100 mL of 0.1 M $ZnCl_2$ solution and 5 mL of 0.5 M NaOH. The mixture was stirred on a magnetic stirrer hot plate at 50 °C for 45 minutes, during which a distinct colour change signified the formation of zinc oxide nanoparticles. The resulting nanoparticles were collected by filtration, dried, and stored for subsequent use [15].

Characterization of Zinc Oxide Nanoparticles and Leaf Extract

The optical properties of the synthesized zinc oxide nanoparticles and the *Balanites aegyptiaca* leaf extract were examined using UV–Visible spectrophotometry over a wavelength range of 200–800 nm. Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify functional groups present in the samples, while X-ray Diffraction (XRD) was used to determine the crystalline structure of the nanoparticles. Scanning Electron Microscopy (SEM) was further utilized to investigate their morphology and particle size.

Antimicrobial Activity

The antimicrobial activity of the synthesized zinc oxide nanoparticles was assessed against selected bacterial and fungal pathogens using the agar well diffusion method. This standard laboratory technique evaluates the capacity of a substance to inhibit microbial growth and is frequently applied in testing the antimicrobial potential of plant- and microbe-derived products. In this assay, a bacterial suspension was uniformly spread over the surface of an agar plate, after which wells were created using a sterile borer. The nanoparticle solution was then introduced into each well, and the plates were incubated under appropriate conditions. Following incubation, clear zones of inhibition surrounding the wells were measured to determine antimicrobial activity. Larger inhibition zones observed with increasing nanoparticle concentrations indicated greater antimicrobial efficacy.

Larvicidal Activity

Larvicidal activity was evaluated following the method of Abba *et al*. [9], with minor modifications. A stock solution of zinc oxide nanoparticles (100 mg/L) was prepared by dissolving 0.1 g of the nanoparticles in distilled water using a 1000 mL volumetric flask. The bioassay involved introducing different larval instars (1st to 4th) into 200 mL plastic cups, with four replicates and a control for each instar, each containing twenty-five larvae. To each

replicate, 100 mL of dechlorinated water was added, followed by the addition of zinc oxide nanoparticles at concentrations of 40 mg/L, 50 mg/L, and 60 mg/L. Larval mortality was recorded, and percentage mortality was calculated using the formula:

$$\text{Percentage Mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of Introduced Larvae}} \text{ X } 100$$

RESULTS AND DISCUSSIONS

Electronic Spectra Results of Zinc Oxide Nanoparticles and Leaf Extract

The electronic spectra of *Balanites aegyptiaca* leaf extract and ZnO nanoparticles are shown in Figure 1A and 1B. ZnO nanoparticles displayed absorbance band at 237 nm and leaf extract shows absorbance band at 282 nm. The shift in the absorbance band to a higher wavelength suggests a reduction in size from bulk molecules to the nanoscale. This shift may result from material transitions, where an electron gains energy and moves from a lower to a higher energy level Perumal *et al*. [16]. This corresponds with the literature of Kiranmai *et al*. [17] and Vijay *et al*. [18] which showed the highest absorbance peaks at around 280nm and 300 and also suggested that, this variation depends on the reducing agent and the type of metal salt used as a precursor. The formation of ZnO nanoparticles in this green synthesis system involves phytochemical-mediated reduction, nucleation, and stabilization processes. Phytochemicals present in Balanites aegyptiaca such as flavonoids, polyphenols, tannins, alkaloids, and proteins play critical roles in reducing Zn^{2+} ions into ZnO nanostructures. These biomolecules contain functional groups such as –OH, –COOH, and $–NH_2$ which donate electrons during the reduction process, thereby facilitating nanoparticle formation (Agarwal et al., 2017; Huq et al., 2023).

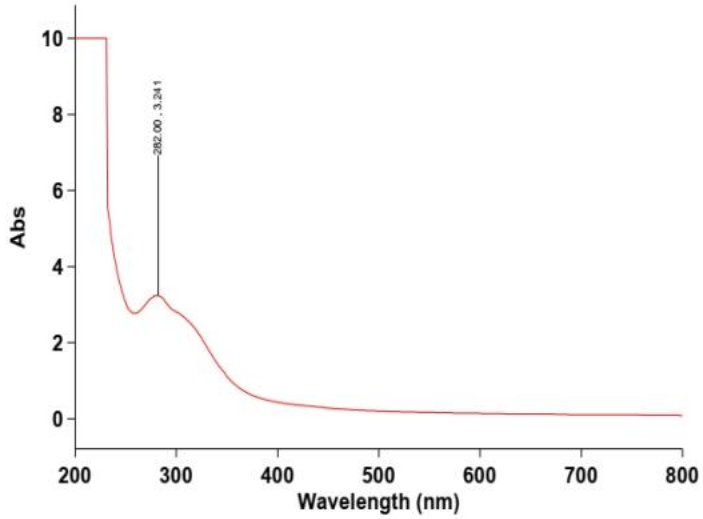

Figure 1A: Electronic Spectra of *Balanites aegyptiaca* Leaf Extract

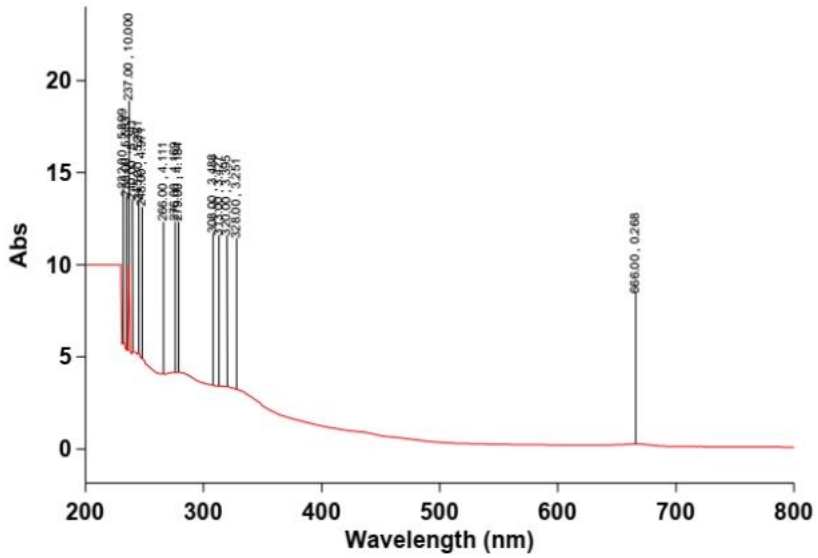

Figure 1B: Electronic Spectra of ZnO Nanoparticles Synthesized Using Leaf Extract

Fourier Transform Infrared (FTIR) Results of Leaf Extract and Zinc Oxide Nanoparticles

The FTIR spectrum of *Balanites aegyptiaca* leaf extract and ZnO nanoparticles are presented in Figures 2A and 2B. The absorption bands observed for leaf extract were 3291 cm^{-1}, 2120 cm^{-1} and 1636 cm^{-1} respectively. The band at 3291 cm^{-1} indicates OH stretching while the band at 2120 cm^{-1} is for C=C alkenes and the band at 1636 cm^{-1} represents C=O stretching. While the ZnO nanoparticles shows the following bands 2996 cm^{-1}, 2914 cm^{-1}, 2094 cm^{-1}, 1435 cm^{-1}, 1312 cm^{-1}, 1043 cm^{-1} and 667 cm^{-1}. These bands are absent in the spectra of the extract, signifying the formation of nanoparticles [19]. The band at 2996 cm^{-1} and 2094 cm^{-1} are due to C-H stretching of aromatic alkanes, 1435 cm^{-1} stretching for C=C in ring aromatic alkenes, 1312 cm^{-1} for C-H bend alkanes, 1043 cm^{-1} stretching for C=N aliphatic amines and 667 cm^{-1} are due to C-Br alkyl halides respectively.

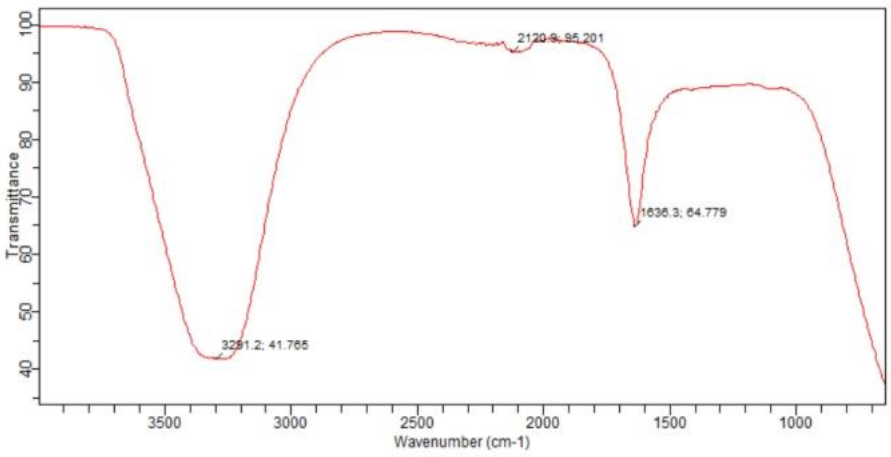

Figure 2A: FTIR spectra of *Balanites aegyptiaca* Leaf Extract

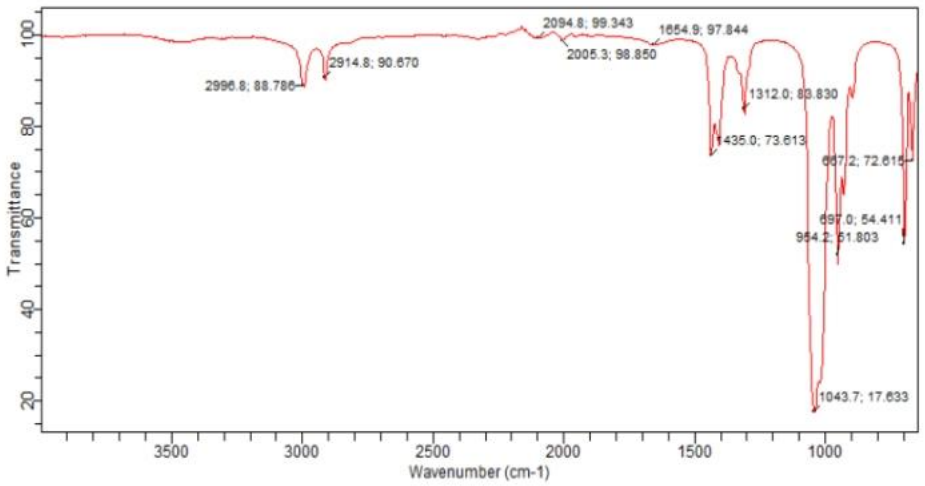

Figure 2B: FTIR Spectra of ZnO Nanoparticles Synthesized Using Leaf Extract

XRD Results of ZnO Nanoparticle Synthesized from The Leaf

The XRD result for the ZnO nanoparticle synthesized from the fruit is presented in Figure 3. It was found that five prominent peaks were observed at $2\theta = 12.32^{o}$, 26.61^{o}, 37.93^{o} ,46.32^{o} and 64.11^{o} with respect to the plane of (110), (101), (201) (210) and (320). It shows hexagonal crystalline structure with the average crystalline size of 38.78nm according to the Scherer equation calculated and it corresponds to the literature reported by Yangma and Jinhou (2020) [20].

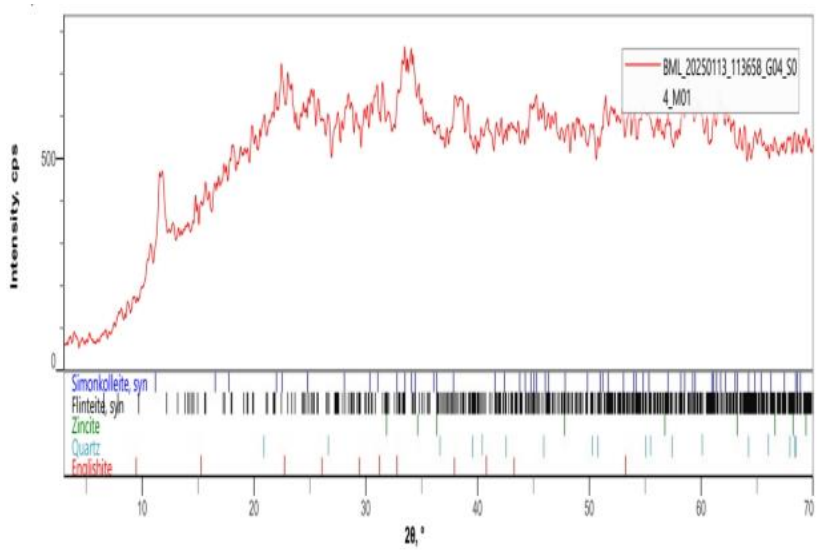

Figure 3: XRD Result of ZnO Nanoparticle Synthesized using Leaf Extract

SEM Analysis of Zinc Oxide Nanoparticle Synthesized from The Leaf

The SEM result for the ZnO nanoparticles synthesized from the leaf is presented in Figure 4. The morphology of the green synthesized zinc oxide nanoparticle shows that the particles have mono dispersed compacted morphology. The result obtained is in agreement with the result reported by Pindiga *et al.* (2022) [21] which shows mono dispersed morphology for the green synthesized copper nanoparticles.

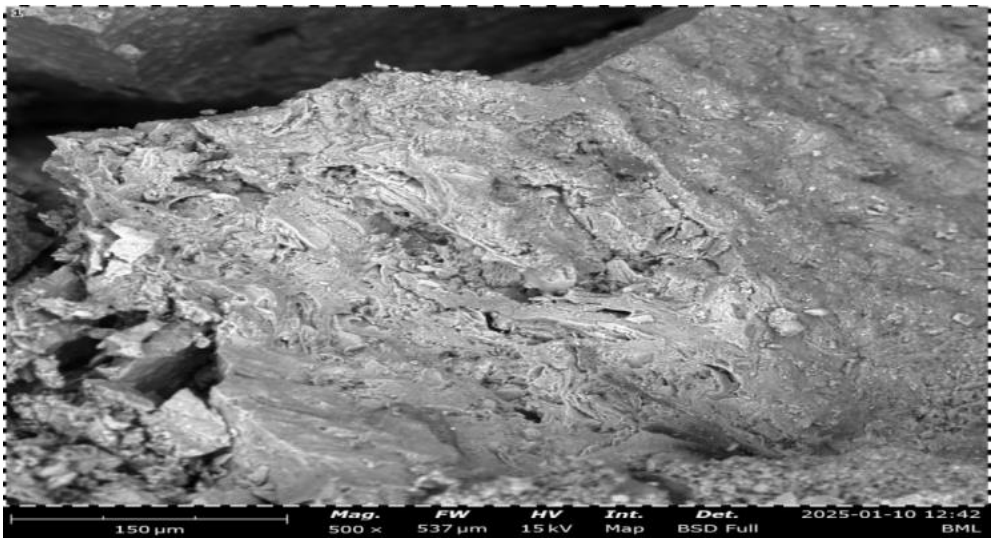

Figure 4: SEM Result of ZnO Nanoparticle Synthesized Using Leaf Extract

Antimicrobial Activity Results

The antimicrobial activity test result for zinc oxide nanoparticle synthesized from the leaf of *Balanites aegyptiaca* are presented in Table 1. The results have shown dose dependent effect across the micro-organisms studied. From the results, the Gram-positive bacteria (*Streptococcus pyogene*) have showed remarkable sensitivity with 18.00±0.00 mm zone of inhibition as compared to standard drug Augmentin (23.00 mm) at the highest concentration of (30 µg/ml). The Gram – negative bacteria (*Klebsiella pneumoniae*) have shown the highest sensitivity with 22.00 mm zone of inhibition which is even greater as compared to standard drug Augmentin (20.00) at the highest concentration of (30 µg/ml). The fungal strain (*Candida albicans*) has showed the least susceptibility effect with (12.00 mm) zone of inhibition compared to the standard drug (fulcin) with 20.00 mm zone of inhibition. The fungal strain (*Aspergillus niger*) has shown resistance across the zinc oxide nanoparticle treated. This corresponds to that obtained by Zacchaeus *et al*. [22] which shows an activity of 29, 18 and 12 mm at concentration 100, 150 and 250 mg/L respectively. The resistance of Aspergillus niger to the synthesized ZnO nanoparticles may be attributed to the complex structural composition of the fungal cell wall. The fungal cell wall consists mainly of chitin, β-glucans, and glycoproteins which form a rigid protective barrier. This multilayered structure significantly restricts the penetration of nanoparticles into the fungal cytoplasm, thereby reducing their antifungal activity (Huq et al., 2023).

Another important mechanism contributing to resistance is the ability of Aspergillus species to produce antioxidant enzymes such as catalase and superoxide dismutase. These enzymes neutralize reactive oxygen species (ROS) generated by ZnO nanoparticles. Since the antimicrobial activity of ZnO nanoparticles largely depends on ROS-mediated oxidative stress, detoxification of ROS significantly reduces their toxicity toward fungal cells (Sirelkhatim et al., 2015).

Table 1: Antimicrobial Susceptibility Test of ZnO-NP of *Balanites aegyptiaca* Leaf

		Concentration (mg/ml)/ Mean Zone of Inhibition in diameter (mm)				
S/N	**Organism**	**30**	**20**	**10**	**Aug. (30µg/ml)**	**Ful. (50µg/ml)**
1	*Staphylococcus aureus*	13.00	08.00	0.00	24.00	-
2	*Streptococcus*	18.00	12.00	7.00	23.00	-

	pyogenes					
3	*Klebsiella pneumoniae*	22.00	16.00	11.00	20.00	-
4	*Salmonella typhi*	17.00	12.00	07.00	22.00	-
5	*Aspergillus niger*	0.00	0.00	0.00	-	21.00
6	*Candida albicans*	12.00	7.00	00.00	-	20.00

Key: Aug = Augmentin, Ful = Fulcin

Larvicidal Test Activity of ZnO Nanoparticle Synthesized from The Leaf

Table 2 shows the larvicidal activity of ZnO nanoparticle synthesized from the leaf of *Balanites aegyptiaca* on Anopheles' larvae. The results indicated that the percentage mortality of the first instar larvae at 40, 50 and 60 mg/L concentrations of ZnO nanoparticle are 92, 96 and 100% respectively. The second instar showed 92, 80 and 96% mortality when tested with 40, 50 and 60 mg/L concentrations of the nanoparticle respectively. For the third instar larvae, the percentage mortality for the 40, 50 and 60 mg/L concentrations were 68, 80 and 92% respectively. While for the fourth instar, the mortality at 40, 50 and 60 mg/L concentrations were 60, 64 and 84% respectively. This agrees with the findings of Bello *et al*. [23] and Shehu *et al*. [24], where they reported a high toxicity of Cu Nanoparticles and ZnO – CuO nanoporous composite against the larvae of anopheles and malaria vector after exposure for 24 hours. Overall, the highest mortality was observed in the first instar (100%) at the concentration of 60 mg/L while the lowest was found in the fourth instar (60%) at 40 mg/L concentration. Murugan *et al*. [25] and Danbature *et al*. [26] reported that the first instar larvae are more susceptible to nanoparticles and the susceptibility decreases with the growing larval instars subjected to the same concentration of nanoparticles. In a similar work reported by Sharon *et al*. [27], the first instar larva of *Aedes aegypti* showed high susceptibility to copper nanoparticles than the 2^{nd}, 3^{rd} and 4^{th} larval instars. This revealed that the more matured the larva, the less susceptible they are to nanoparticles. This is due to the fact that most juvenile larvae are more delicate and therefore easily intoxicated by nanoparticles [9]. The calculated LC_{50} values were 36.24, 36.04, 37.38 and 38.07 mg/L for the first to fourth instars larvae respectively. The findings of Bello *et al*. [23] revealed that the larvicidal activity of bio – synthesized Copper nanoparticles of African spinach on Anopheles' larvae (LC_{50} = 47.20 mg/L), a higher LC_{50} compared to the findings of the present work. On the other hand, Rawani *et al*. [28] reported the larvicidal activity of Ag Nanoparticles which showed LC_{50} value of 1.59 ppm for berries dry leaf and fresh leaf against the larvae of *Anopheles stephensi*. These variations may be attributed to several factors such as the reducing agent (species of plant or parts) used in the synthesis, the type of metal and the mode of action [9]. The mortality rate recorded in this work showed that the activity of the ZnO nanoparticle synthesized

from the leaf of *Balanites aegyptiaca* on the Anopheles Mosquito larvae is dose-dependent as reported by other works [29], [25], [30], [31], [26], [9] and [24].

Table 2: Larvicidal Activity of ZnO Nanoparticle Synthesized from The Leaf Against first to fourth Instars Larvae of Anopheles Mosquito

		Conc.	% Mortality	LC_{50}	
Anopheles	**S/N**	**(mg/L)**	**At 48 Hours**	**(mg/L)**	**R^2**
First	1	40	92		
Instar	2	50	96	36.24	0.981
	3	60	100		
Second	1	40	92		
Instar	2	50	80	36.04	0.929
	3	60	96		
Third	1	40	68		
Instar	2	50	80	37.48	0.912
	3	60	92		
Fourth	1	40	60		
Instar	2	50	64	38.07	0.837
	3	60	84		

Conflict of interest: The authors declare that there is no conflict of interest

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