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Biochemical changes caused by Zinc oxide Nanoparticles synthesized using Ficus thonningii extract in induced diabetic Wistar Albino Rats

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Abstract*— Green synthesis of nanoparticles known as the synthesis of nanoparticles using biosynthetic methods involving naturally reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract. The synthesis of nanoparticles by the use of biological methods have reached a colossal signification above physical and chemical procedures, this is due to the use of innocuous, biocompatible, and ecologically-sound substrates and remarkably uncomplicated synthetic processes at encompassing conditions. In this research, the synthesis, characterization and the determination of the antibacterial activity of Zinc oxide Nanoparticles (ZnO NPs) was ascertained using stem bark extract of Ficus thonningii (Blume) as a stabilizing agent. However, the anti-diabetic activity and some biochemical changes caused by the synthesized ZnO NPs on alloxan-induced diabetic Wistar Rats were also determined. ZnO NPs were synthesized using biological method at different concentrations of ZnO solution (1mM, 2mM, 3mM and 4mM), these NPs were characterized using UV-visible spectroscopy, FTIR and SEM. Wistar rats weight 185 ± 5g were grouped into nine (9) groups (A, B, C, D, E, F, G, and I). Group A served as normal control which was given only feed and water, group B, C, D and E, were induce and treated with 1mM, 2mM, 3mM and 4mM ZnONPs respectively, then F and G were induced and treated with aqueous extract and ethanolic extract while H was induced and treated with Glibenclamide (standard drug for diabetes) by gavage method and finally I was induced and untreated which served as diabetic control. The results showed that ZnO NPs were synthesized with the indication of the change in colour from yellow to dark brown colour. The UV-visible spectroscopy was taken at range between 200nm to 700nm which displayed different peaks at the range of 209nm to 383nm. However, the FTIR showed the existence of various functional groups such as C=C stretch, C≡C stretch and Alcohol OH stretch representing the bioactive compounds such as phenol, amine and many others. The Nanoparticles were analyzed with SEM to examine the morphology of the Nanoparticles. The diabetic induced rats revealed significant decrease in fasting blood glucose after treatment compared with the diabetic untreated rats, the doses were effective when compared with Glibenclamide treated rats. The levels of serum Alkaline phosphatase, Alanine Amino transferase, Albumin, Globulin, Bilirubin, Total Protein, Urea, Creatinine and Electrolytes concentrations displayed no significant increase relative to diabetic control ($p < 0.05$; $n \geq 5$).

***Index Terms*— biomedical applications, kidney, liver, stabilizing agents, diabetes, nanoparticles**

INTRODUCTION

Nanotechnology refers to science and engineering involved in the design, manipulation, synthesis, characterization and application of nanoscale materials, systems, devices and structures. This technology is an opening to new medicinal restorative changes for many media that cannot be utilised successfully as traditional concepts because of their poor functionality [1]. Nanotechnology as an emanating rapidly expanding field with its implementation in Science and Technology for the purpose of discovering advanced materials at nano scale level [2]. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision in manufacturing of materials at the nanometer level [3].

Nanobiotechnology as a discipline in Nanotechnology has become prominent in research and development at the atomic, molecular, or macromolecular levels in the range between 1nm to 100nm to produce structures, devices, and systems that have innovative functional properties an intersection derived from nanotechnology and biology [4] It is the integration of multiple disciplines such as biotechnology, nanotechnology, chemical processing, physical methodology and system engineering into biochips, molecular motors, Nano crystals and Nano biomaterials [5].Nano biotechnology deals with synthesis, design and stabilization of various particles at nano scale using biological tools and techniques, it involves manipulation of particles structure with dimension smaller than 100 nm [6, 7].

Nanoparticles are collection of atoms in the size range of 1-100 nm. The use of nanoparticles is gaining priority in the present years as they possess defined chemical, optical and mechanical properties [8]. Nanoparticles have been classically produced by physical and chemical methods, involving techniques like heating and irradiation [9]. However, these methods are high-priced, noxious and unsafe, thus the necessity for switch in approaches that are harmless,

low cost, free of contaminants for biological and medical applications, more secured methods referred to as green methods of nanoparticles synthesis. These methods have shown encouraging solutions to those constituted by classical approaches. However, the green synthesis of ZnO NPs using variant biological materials such as spider cobweb, cola nitida (kolanut) seed, seed shell, pod and cell-free extract of Bacillus safensis with dominant biological activities was recently demonstrated [10].

Zinc an enzyme activator predominantly exists in all body tissues such as brain, muscle, bone, skin and many more as a vital trace element. As the main component of various enzyme systems, Zinc plays a part in various metabolic processes like retaining the structural integrity of insulin, significant roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis [11]. Zinc oxide nanoparticles (ZnO NPs), as one of the most vital metal oxide nanoparticles, are widely utilized in numerous fields due to their peculiarities in both physical and chemical possessions [12]. Zinc oxide nanoparticles (ZnO NPs) are used in an expanding number of industrial products such as rubber, paint, coating, and cosmetics. In the past two decades, ZnO NPs have become one of the most popular metal oxide nanoparticles in biological applications due to their excellent biocompatibility, great economic, and harmless effect. Furthermore, ZnO NPs have emerged as a propitious potential in biomedicine, especially in the fields of anticancer, anti diabetic and antibacterial fields, which are involved with their powerful ability to trigger excess reactive oxygen species (ROS) production, release zinc ions, and induce cell apoptosis. In addition, ZnO NPs are applied in the rubber industry an anti-wearing for the rubber composite, improve performance of high polymer in their toughness and intensity and anti aging, and other functions [13]. The strong UV absorption properties in ZnO increased the use in personal care products, such as cosmetics and sunscreen [14]. In addition, ZnO NPs have supercilious antibacterial, antimicrobial and magnificent UV blocking properties. Therefore, in the textile industry, the completed fabrics exhibited the attractive functions of ultraviolet and visible light resistance by adding ZnO NPs [15]. Apart from the applications afore-mentioned, Zinc oxide can also be used in other branches of industry, including concrete production, photo catalysis, electronics, electro technology industries, and many others [16]. Moreover, ZnO NPs displayed an outstanding luminescent property which turned out to be one of the dominant candidates for bio imaging. Here, the synthesis and recent advances of ZnO NPs in the biomedical fields was summarized, which will be helpful for facilitating their future research progress and focusing on biomedical fields [17].

Ficus thonningii commonly known as the wild fig. is a multi-stemmed, evergreen or briefly deciduous tree with a dense, rounded to spreading crown, and is mainly distributed in the upland forests of tropical and subtropical Africa, at altitudes of between 1,000–2,500 m and grows best in light, deep and well drained soils [18] Ficus thonningii, is one of the many fruit-bearing trees that have traditionally been used for treating diseases in Africa and beyond, it is also used in ethnomedicinal systems, F.thonningii is a well-known ornamental tree that is also used in improving agroforestic systems. Its leaves are used as fodder and its bark is used for making bark cloth. Like many woody trees, F.thonningii is commonly used in homesteads for fencing, firewood and construction [19]. The leaves are alternate or whorled, mid-dark green and sub glossy above whilst paler below, and can be rounded or tapering, 4.5-12 cm long, hairless or finely hairy with a prominent midrib [20]. F. thonningii is a flowering tree that is pollinated by wasps which enjoy a symbiotic relationship and live in the syconium of its fruit and it can easily be propagated using seeds and cuttings [21]. All parts of F. thonningii are medicinally useful, Macerations of fresh F.thonningii leaves, taken orally, have been used by traditional healers for treating diarrhoea, gonorrhoea and diabetes mellitus [22]. In Angola decoctions of F.thonningii leaves are used for treating wounds. Leaf extracts are also used for treating bronchitis and urinary tract infections [23]. A decoction of the leaves is used in Mali for treating urinary schistosomiasis [24]. In Nigeria, a maceration of the leaves is used for treating stomach pains, gastritis, gastric ulcers and other stomach conditions in animals [25]. The leaves can also be used for treating liver disorders and disease conditions associated with jaundice [26]. Other medicinal uses of the leaves reported include treatment of bone movement disorders, ringworm, thrush, scabies and athlete's foot. The latex has been traditionally used for treating fever, tooth decay and ringworm cataract in the eye [27], also as a vermifuge. Traditionally, the stem bark is pounded and the infusion used for treating influenza, sore throat, colds, arthritis, rheumatism and to relieve inflammation [28]. In Tanzania, it is also used to stimulate lactation and in Mali and Senegal, to treat respiratory diseases such as emphysema. The roots are traditionally used for the treatment of malaria, fever, hepatitis and dental pains. Further down, in Central Africa, Congo Brazaville, the bark is used for treating diarrhoea, cysts, skin diseases and ulcers. Additionally, relieving stomach pains, diarrhoea, pneumonia and chest pains are treatments exerted by the roots. Other people use the bark decoction to enhance fertility and induce the menstrual cycle [29]. In Southern Africa it is used as relief for constipation and bowel disorders [30].

Furthermore, a large number of people suffer from diabetes all over the world which is a metabolic disorder characterized by high blood glucose. The development and innovation of multiple modes of action in the medication of diabetes is very vital because of the increasing number of the patients annually [31]. Recent researches have demonstrated

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the role of metals in glucose metabolism and deficiencies with diabetes, example of these metals include copper, magnesium and zinc [32].

Moreover, most enzymes are indicators of diseases and its state reported by various researchers and diagnosis. Increase in the levels of kidney and liver enzymes is an indicative sign of cell or tissue damage. Thus, it is very crucial to conduct researches on these enzymes (such as AST, ALP, ALT) to ascertain the proper functions of some key organs in the body [33]. Creatinine as a significant constituent of the muscle is excreted through the glomerular filtration in the kidney, neither re-absorbed, secreted nor synthesized but its clearance is equal to GFR. However, urea as the end product of metabolism in protein biosynthesis is produced in the liver which migrate to the blood and is excreted by the kidneys. These metabolic processes and many others require a functional organ to take place efficiently [34].

In this research, the green synthesis of ZnO NPs using Ficus thonningii stem bark extract was used as the reducing and stabilizing agent. The characterizations of the synthesized nanoparticles using UV-visible spectroscopy, FTIR and SEM were reported while

the activity of the synthesized nanoparticles was examined against a gram positive and gram negative bacteria (Streptococcus aureus and Salmonella typhi) using Gentamicin as control, the antidiabetic effect of the synthesized nanoparticles was evaluated using Glibenclamide as the judge using the alloxan-induced diabetic rats, checked the changes in some biochemical parameters caused by the synthesized nanoparticles.

MATERIALS AND METHODS

Chemicals and Reagents

The reagent and apparatus used are of analytical grade obtained from Kano University of Science and Technology Wudil. The apparatus were washed thoroughly dried in an oven and stored for further analysis.

Sample collection and Preparation of extract

The stem barks were collected from Ajingi Local Government Area, Kano State and authenticated by the Department of Plant Biology, Bayero University Kano, Nigeria with a voucher number BUKHAN 0110 and was deposited at the herbarium of the Institute. The stem bark from the plant Ficus thoninngi, was dried under shade and pulverized into coarse powder using mortar and pestle.

The extract was prepared using the method previously described by Suresh et al [35]. Briefly, the extraction was performed using water as a solvent in which 20g of the powder was soaked in 100mL deionized water in a conical flask and heated in a water bath under constant shaking at 45 ◦C 24 hours, filtered with a Whatman No 1 filter paper and the filtrate was stored at 4 ◦C until used.

Synthesis of Zinc oxide Nanoparticles (ZnO NPs)

ZnO NPs using F.thonningii aqueous stem bark extract was prepared using the method described by Elham Zare et al. with some minor modifications [36]. Synthesis of ZnO NPs was carried out using 1mM, 2mM 3mM and 4mM Zn(NO3)2•6H2O solutions in 90mL distilled water, 10mL of F.thonningii extract was added dropwise to the mixture under constant stirring at 60◦C for five(5) consecutive hours for complex formation . After the complex was formed, the mixtures were calcined for 2 hours at 350 ◦C in a muffle furnace.

Characterization of ZnO Nanoparticles (ZnO NPs)

The produced nanoparticles were characterized by UV Vis spectroscopy using (LAMBDA 25/35/45). UV-spectrophotometer, and a sensitive technique Fourier Transform Infrared (FTIR) spectroscopy analysis was carried out using IR Affinity-1S spectrophotometer (Shimadzu, UK) on the powder sample of ZnO NPs. The ZnO NPs solution was centrifuged at 10,000 rpm for 20 min. The solid residue obtained was then dried at room temperature, and the powder obtained was used for FTIR measurements using KBr pellets. The nanoparticles were then analyzed with Scanning Electron Microscope (SEM) which was done using LEO 1430 VP, SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Antibacterial Activities of the Synthesized ZnO NPs

The bacterial activity of the produced ZnO NPs was analysed using agar well diffusion method Perez et al [37]. The antibacterial activity test was done on Bacteria using gram positive and gram negative (Staphylococcus aureus and Salmonella typhi). Each bacterium was grown in peptone water, and an 18-hour culture was used to seed plates of Mueller-Hinton Agar. The plates were then bored using a cork borer (6 mm) to create wells. The wells were irrigated with 100l of graded concentrations of ZnO NPs prepared by dispersion in sterile distilled water. The plates were thereafter incubated at 37oC for 24 hr. At the end of incubation, the plates were examined for zones of inhibition.

Minimum Inhibitory Concentration (MIC) of ZnO NPs

The MIC of the F.thonningii ZnO NPs was determined by broth dilution method. Different test tubes were labeled and 5ml of nutrient broth was introduced into each test tube, 0.5 ml of bacteria suspension was inoculated. This was followed by the addition of the extract to the sterile nutrient broth test tubes and incubated at 37oC for 24 hours. In the control tubes, only extract was not added (contains nutrient broth +

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bacteria), the other control contains (nutrient broth + nanoparticles). By comparing the three sets of tubes, the uninoculated test tubes were used to check the sterility of the medium and as negative control while the positive control tubes were used to check the suitability of the medium for growth of the microorganisms. The MIC was determined by the lowest concentration of the extract that prevented visible growth [38].

Minimum Bactericidal Concentration (MBC) of ZnONPs

The MBC of the extract was determined by sub culturing the contents of the tubes that showed inhibition of growth due to the presence of extract (nanoparticles). The tubes were plated out on nutrient agar plates which had neither antibiotics nor extract and incubated for 24 hours to determine whether there is growth of microorganisms or not, to confirm the effect the extract (nanoparticles) on the bacteria.

Experimental Animals

Albino Wistar rats weighed 185 ± 5 g from the Biological Science Department's Animal House, Bayero University, Kano. The Animals were kept in cages in the experimental Animal House and allowed to acclimatize for two weeks before the commencement of treatments. Animals were maintained under standard hygienic and environmental conditions with alternate 12h light and dark cycle and were served with food and clean water. Handling of animals was consistent with relevant guidelines on the care and use of laboratory animals (National Research Council 2011). They were sustained throughout the experimental period in conformity with the Ethics Committee of Kano University of Science and Technology, Wudil.

Experimental Design

Wistar rats weight 185 ± 5g were grouped into nine (9) groups (A, B, C, D, E, F, G, H and I). Group A served as normal control which was given only feed and water, group B, C, D and E, were induce and treated with 1mM, 2mM, 3mM and 4mM ZnO NPs respectively, then F and G were induced and treated with aqueous extract and ethanolic extract while H was induced and treated with Glibenclamide (standard drug for diabetes) and finally I was induced and untreated which served as diabetic control.

Blood Sampling and Tissue Preparation

The rats were weighed, anesthetized with 60/6 mg/kg of ketamine/xylazine intraperitoneally 24h after the last treatment. Blood samples were taken in a clean and sterile sample container. The serum was obtained after the blood sample was centrifuged at 3000 rpm for 15 min stored at −80◦C prior to analysis.

Biochemical Parameters Analysis

The serum levels of AST, ALT, ALP, ALB, BIL, CREA, urea, serum electrolyte concentration, (Randox Laboratory Limited County Antrim, United Kingdom) according to the manufacturer's instructions. The protein content of the serum was determined by Biuret method as described by [39].

Statistical Analysis

Statistical analysis of the data was carried out through the Statistical Package for Social Science (SPSS) computer software version 11 using ANOVA and student's t-test at 95% confidence limit with P-value of (<0.05) being considered as significant. Results were expressed as mean ± standard deviation (mean ± SD).

RESULTS AND DISCUSSION

In this present study, ZnO NPs were synthesized using F.thonningii stem bark extract as bioreductant. The average weight of the experimental rats before and after induction, two (2) weeks after treatment and four (4) weeks after treatment is given in Table 1 in grammes. It can be observed that the weight of all groups increased when compared with untreated group of rats.

UV visible spectroscopy as one of the most commonly used technique for the characterization of synthesized nano particles was used to analyzed the formation of ZnO NPs from Zinc nitrate solution, thus the color change from dark brown to light brown formed indicates the formation of ZnO NPs.

The results of the UV-visible absorption of the synthesized ZnO NPs with different concentrations 1mM, 2mM, 3mM and 4mM displayed different absorption peaks between 209nm to 383nm as shown in Figure 1A, B, C and D which is in conformity with the range of light absorption maxima scanned at range of 200-700nm. Umar et al reported the biosynthesis of ZnO NPs using Albizia lebbeck stem bark extract and Zinc nitrate with absorption peak at 368nm [40]. The increase in the concentrations of the metal ions of Zinc nitatre unfolded the effect on the intensity of the synthesized nanoparticle and gives a reflection of the stability of the synthesized ZnO NPs after 24hours as shown in Figure 1 for 1mM, 2mM, 3mM and 4mM when light passes through the solution, the confirmation of nanoparticles was displayed in the range between 200 to 400nm.

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Table 1: The average weight of experimental Rats before and after induction, weeks after treatment

Experimental groups	Average weight before induction (g)	Average weight after induction (g)	Average weight 2 weeks after treatment (g)	Average weight 4 weeks after treatment (g)
A- Normal Control	182±3	181±4	189±7	197±4
B- 1mM ZnO NPs	189±3	188±5	190±4	193±3
C- 2mM ZnO NPs	187±2	187±3	189±3	197±2
D-3mM ZnO NPs	187±5	185±3	188±6	197±2
E- 4mM ZnO NPs	184±4	183±2	190±4	198±4
F- Aqueous Extract	186±2	184±4	187±3	199±2
G- Ethanolic Extract	185±6	184±4	188±6	190±3
H- Glibenclamide	190±5	188±5	193±6	197±2
I- Diabetic untreated	181±2	179±4	176±8	167±7

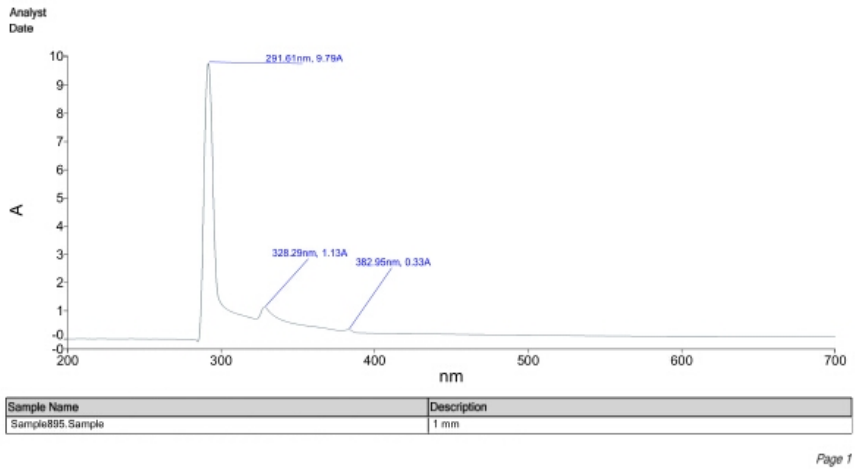

Figure 1: The UV-vis absorption spectrum of the biosynthesized ZnO NPs for 1mM.

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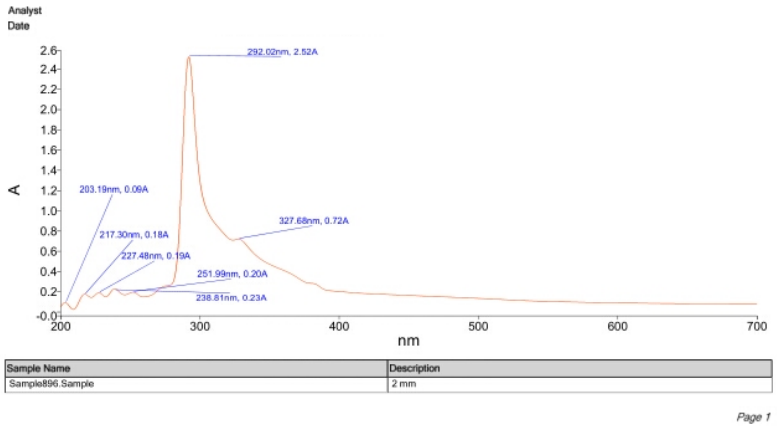

Figure 1: The UV-vis absorption spectrum of the biosynthesized ZnO NPs for 2mM.

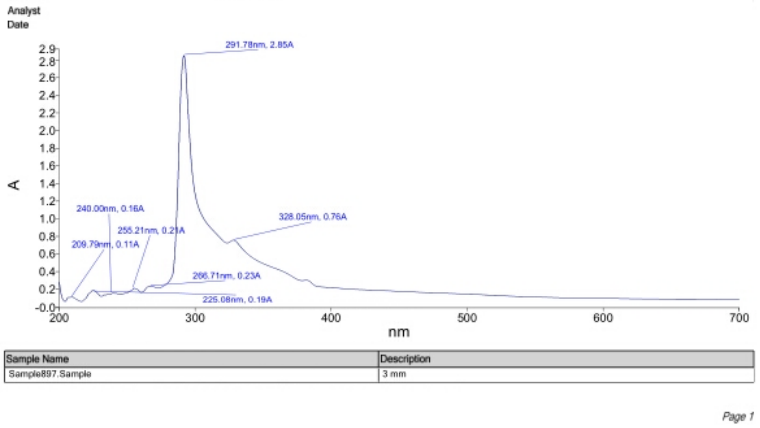

Figure 1: The UV-vis absorption spectrum of the biosynthesized ZnO NPs for 3mM.

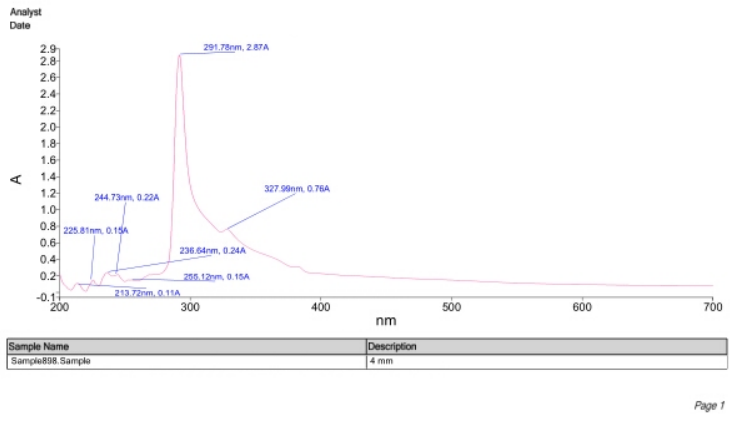

Figure 1: The UV-vis absorption spectrum of the biosynthesized ZnO NPs for 4mM.

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FTIR result shown in the Table 2 displays some biomolecules and functional group that are responsible to stabilized and capped the synthesized ZnONPs at concentration of 4mM, 3mM, 2mM, and 1mM. Such functional groups are pointed within a distinctive frequency which recorded the FTIR spectrum as shown in Figure 4, 5, 6, 7 and 8. The spectrum consists of four distinct peaks in the entire range of recorded spectrum at both 4mM, 3mM, 2mM and 1mM synthesized ZnO NPs. Bands at 1640 cm-1 are attributed to C=C bending of alkenes, N-H bending of primary amine and C=O stretching vibration of carbonyl of amide [41] The band at 2117,2124, and 2125 cm-1 as they are within the range of 22032114 cm-1 may denote the stretching C≡C stretch stretch bonds found in alkynes [42] whereas a band noticed at 3257,3253 and 3260 cm-1 as they are within the range of 3600-3200 cm-1 indicates the O-H stretching of aromatic compounds (like phenol) [43].

Table 2: FTIR Result showing different functional groups and intensities

Concentrations	Frequencies (Cm^{-1})	Functional group	Intensity	Assignment
4mM				
	1640	C=C stretch	Strong	Alkene
	2132	C≡C stretch	Variable	Alkyne
	3260	O-H stretching	Strong	Alcohol
3mM	1640	C=C stretch	Strong	Alkene
	3260	O-H stretch	Strong	Alcohol
2mM	1640	C=C stretch	Strong	Alkene
	3268	O-H stretch	Strong	Alcohol
1mM	1637	C=C stretch	Strong	Alkene
	3264	O-H stretch	Strong	Alcohol
	3819	O-H stretch	Strong	Alcohol
	3838	O-H stretch	Strong	Alcohol

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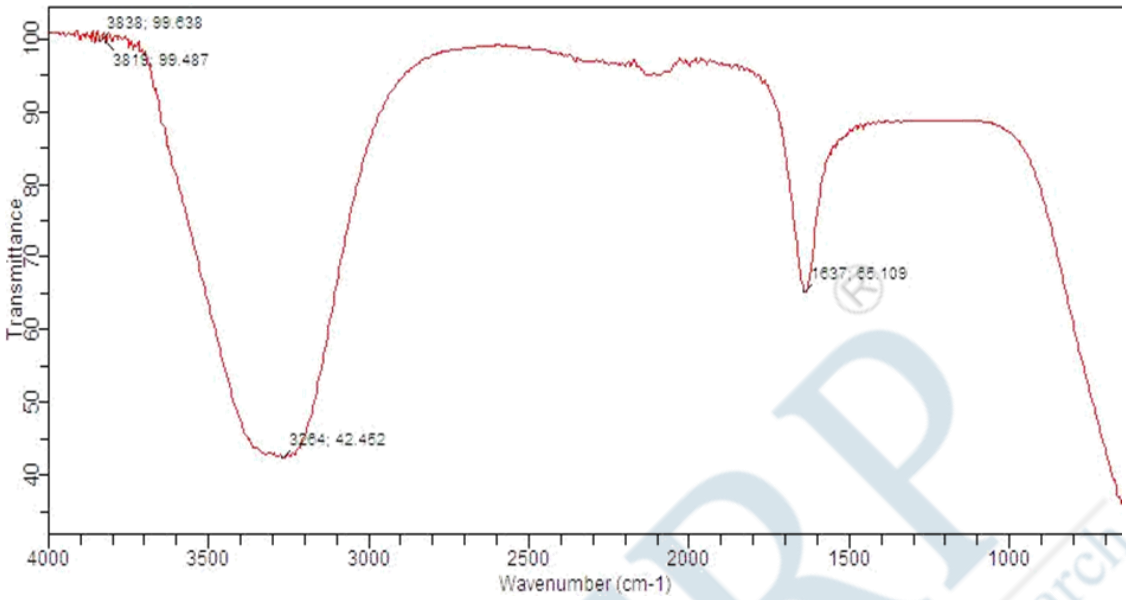

Figure 2. The FTIR result of the biosynthesized ZnO NPs for 1mM.

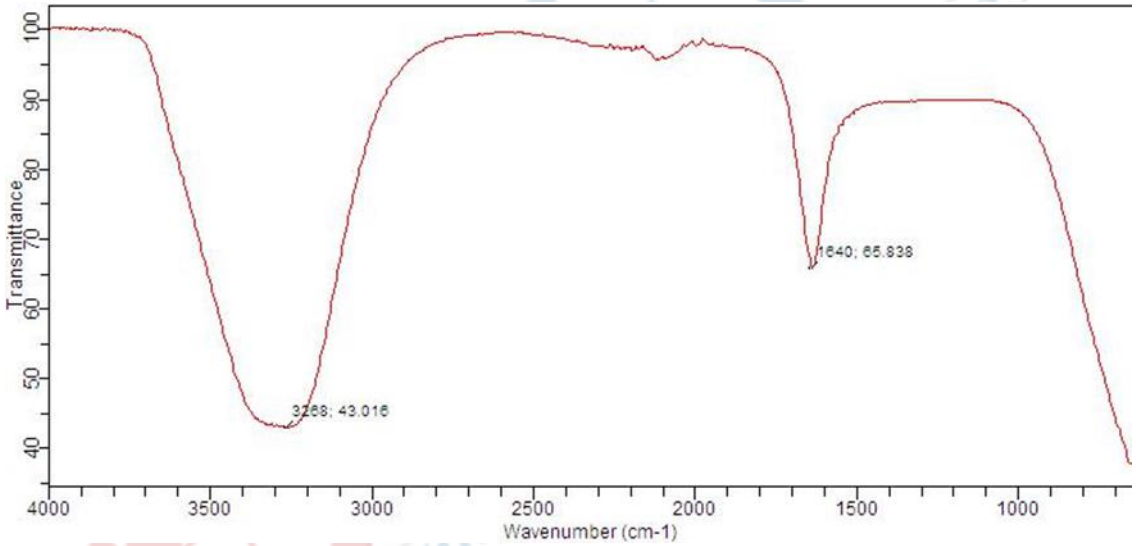

Figure 3: The FTIR result of the biosynthesized ZnO NPs for 2mM.

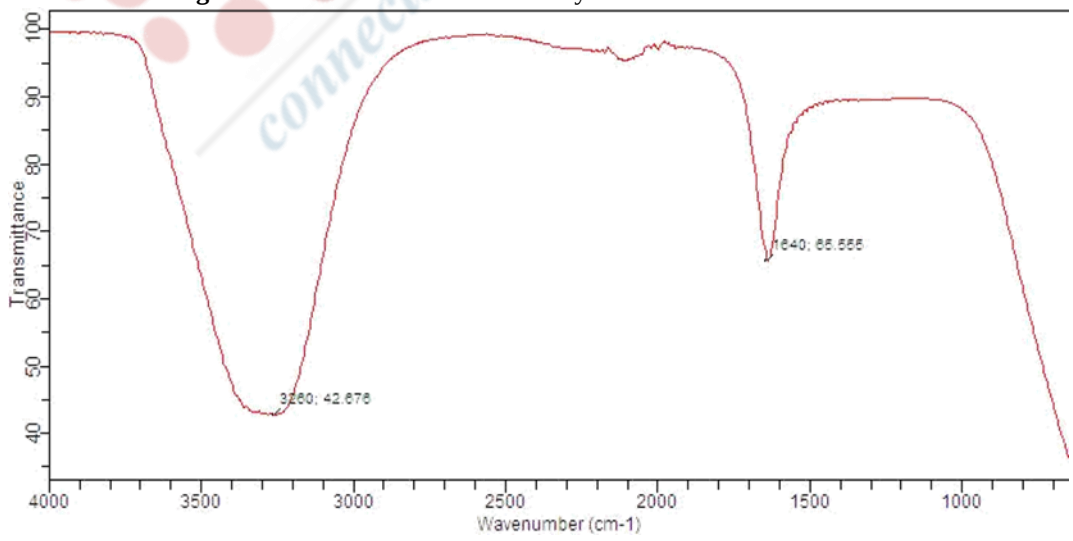

Figure 4. The FTIR result of the biosynthesized ZnO NPs for 3mM.

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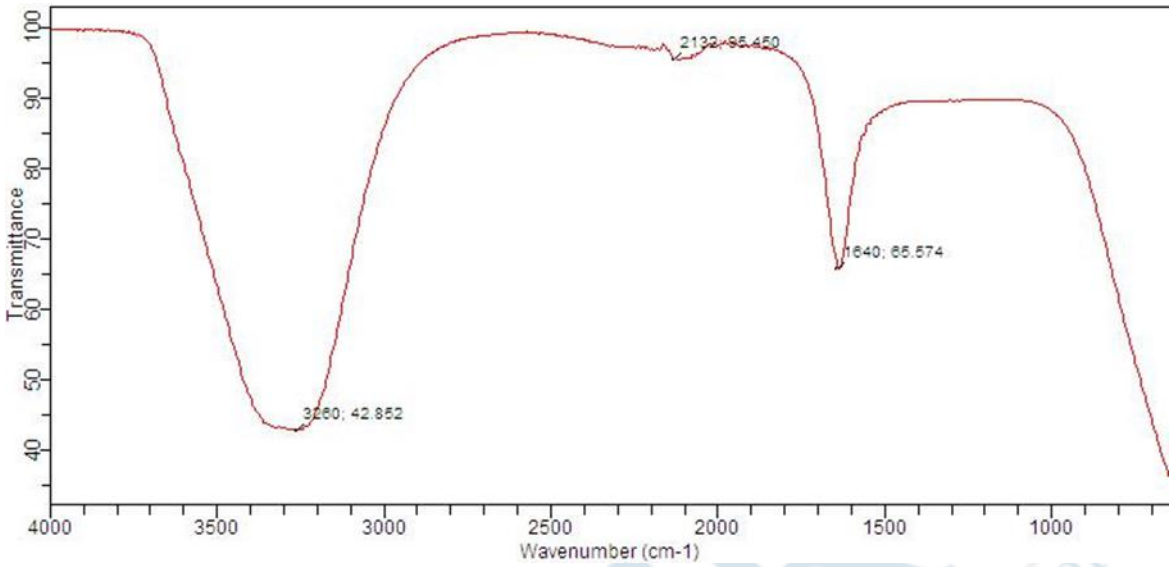

Figure 5. The FTIR result of the biosynthesized ZnO NPs for 4mM.

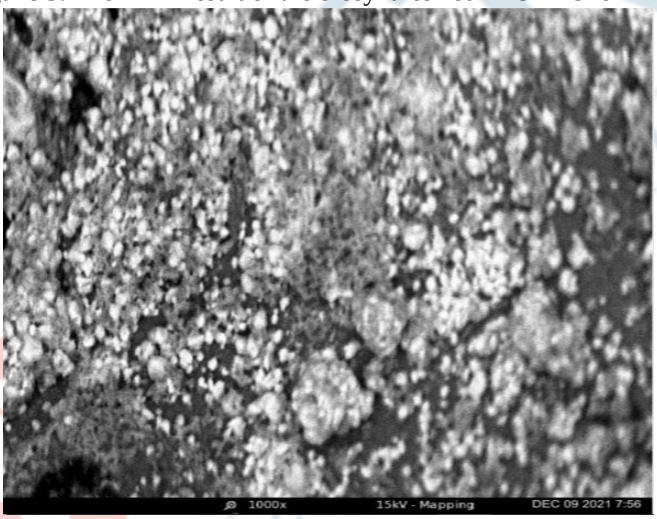

Figure 6A: The SEM micrograph obtained for 1mM ZnO NPS

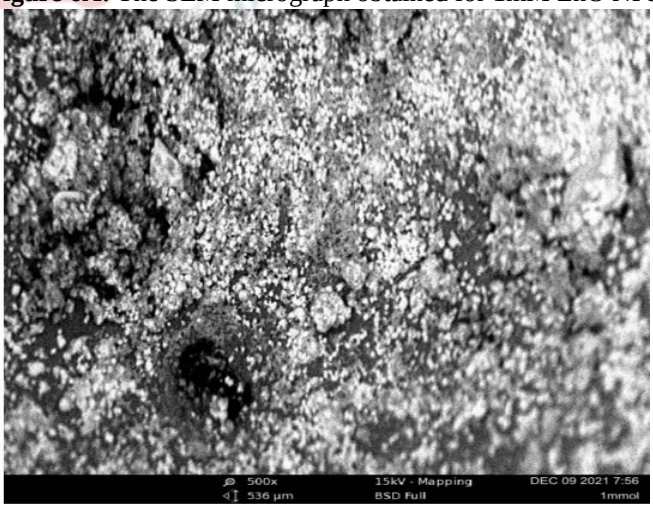

Figure 6B: The SEM micrograph obtained for 1mM ZnO NPS

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Figure 6C: The SEM micrograph obtained for 1mM ZnO NPS

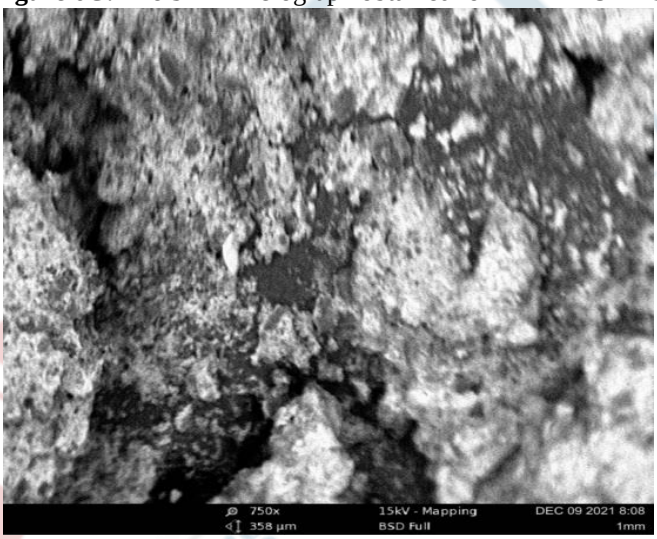

Figure 6D: The SEM micrograph obtained for 1mM ZnO NPS

However, Scanning Electron Microscopy (SEM) analysis of the synthesized 1mM ZnO NPs (Figure 6) confirms the existence of small-scale particles which displayed the surface morphology of the ZnO NPs with many irregular morphology due to slight accumulation of Zinc oxide. Umar et al synthesized ZnO NPs using Albizia lebbeck extract which revealed irregular spherical morphology at the concentration of 0.1M, 0.05M and 0.01M with various accumulated particles [40].

ANTIBACTERIAL ANALYSIS

The antibacterial activity of the synthesized ZnO NPs was carried out using gram +ve and gram -ve bacteria (Staphylococcus aurens and Salmonella typhis) respectively. Measurement of zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration ware carried out to determine the antibacterial efficacy of the synthesized ZnO NPs in the presence of Ciprofloxacin as control (Table 3). Table 3 shows the Zone of inhibition of the synthesized ZnO NPs Nanoparticles at different concentration and control (ciprofloxacin). It can be examined that the diameter of inhibition zone is lower for gram negative bacteria than that of gram positive bacteria and the efficacy of the synthesized ZnO NPs is increasing as the concentration of the ZnO NPs is increased.

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Table 3: Zone of inhibition (mm) of ZnONPs Synthesized from aqueous stem bark extract of Ficus thonningii at different concentrations and control (Gentamicin)

Test Organisms	ZnONPs 1mM	ZnONPs 2mM	ZnONPs 3mM	ZnONPs 4mM	Control
Staphylococcus aureus	14 ± 0.2	16 ± 0.4	16 ± 0.8	19 ± 0.2	20 ± 0.
Salmonella typhi	10 ± 0.6	11 ± 0.2	15 ± 0.4	17 ± 0.8	15 ± 0.2

replicate experiments and analyzed using one-way ANOVA.

Data are presented as the mean ± SD of at least n ≥ 5

Table 4: MIC results of ZnONPs Synthesized from aqueous stem bark extract of Ficus thonningii at different concentrations and control (Gentamicin)

Test Organisms	ZnO NPs 1mM	ZnO NPs 2mM	ZnO NPs 3mM	ZnONPs 4mM	Control
Staphylococcus aureus	-	-	-	-	-
Salmonella typhi	-	-	-	-	-

growth

Key = Negative (-) No growth, while Positive (+) there is

Table 5: MBC results of ZnONPs Synthesized from aqueous stem bark extract of Ficus thonningii at different concentrations and control (Gentamicin)

Test Organisms	ZnONPs 1mM	ZnONPs 2mM	ZnONPs 3mM	ZnONPs 4mM	Control
Staphylococcus aureus	-	-	-	-	-
Salmonella typhi	+	-	-	-	-

Key = Negative (-) No growth , while Positive (+) there is growth

Antidiabetic Effect of synthesized ZnO NPs

The evaluated result of this present study after 4 weeks of treatment with the synthesized ZnO NPs revealed decreased in the levels of fasting blood sugar levels (FBS) in Table 6 relative to control, this is similar to the report of Alkaladi et al [44].

Table 6: The result of Fasting Blood Sugar for the duration of 4 weeks

Experimental groups	FBS (mg/dL) Before induction	FBS (mg/dL) After induction	FBS (mg/dL) After 1 week Treatment	FBS (mg/dL) After 2 weeks treatment	FBS (mg/dL) After 3 weeks treatment	FBS (mg/dL) After 4 weeks Treatment
Normal	111.2 ± 5.8	175.3 ± 2.7	117.0 ± 5.9	91.9 ± 2.3	81.7 ± 2.3	77.9 ± 5.9
1mM ZnO NPs	113.9 ± 7.9	190.9 ± 3.7	131.1 ± 6.3	105.3 ± 6.7	95.9 ± 5.9	85.0 ± 4.9

2mM ZnO NPs	90.0 ± 13.1	223.4 ±5. 9	129.0 ± 10.1	102.9 ± 5.7	97.9 ± 7.6	90.9 ± 3.7
3mM ZnO NPs	90.0 ± 13.1	266.9 ± 6.4	119.8 ± 3.9	111.0 ± 9.4	96.0 ± 4.2	92.2 ± 6.5
4mM ZnO NPs	95.9 ± 7.2	337.9 ± 4.5	115.7 ± 4.7	97.6 ± 5.8	88.0 ± 3.2	80.0 ± 5.6
Aqueous extract	97.6 ± 1.3	176.2 ± 3.7	102.6 ± 1.9	93.9 ± 5.4	82.1 ± 1.8	80.9 ± 4.3
Ethanolic extract	100.4 ± 3.6	218.7 ± 5.4	117.2 ± 2.3	110.6 ± 9.8	103.0 ± 2.8	99.7 ± 3.2
Glibenclamide	90.80 ± 5.2	195 ± 6.8	152.5 ± 7.6	136.6 ± 11.4	119.8 ± 5.7	105.5 ± 9.7
Diabetic Untreated	96.1 ± 5.3	219.2 ± 3.5	245.7 ± 5.70	282.9 ± 4.80	286.2 ± 7.5	323.4 ± 6.8

Biochemical Parametres Analysis

This report studied the effect of different concentrations of ZnO NPs on serum level of kidney and liver markers reported in Tables ,7,8 and 9. There is no significant change observed relative to control as compared to the report of Kavaz et al 2021 [45].

Table 7: Results of Biochemical parameters analysis

Experimental Group	ALP	AST	ALT	T. BIL
Normal	27 ± 0.6	2±0.6	6± 0.354	9 ± 1.061
1mM ZnO NPs	1±0.5	16 ± 0.6	2± 0.566	4 ± 0.990
2mM ZnO NPs	13.3 ± 0.9	2.2 ± 0.9	2.0 ± 0.01	5 ± 0.01
3mM ZnO NPs	13 ± 0.5	10 ± 0.5	44 ± 0.354	4 ± 0.071
4mM ZnO NPs	8.0 ± 0.9	16.2 ± 0.2	2.0 ± 0.02	3.1 ± 0.07
Aqueous extract	1±0.3	6±0.3	2 ± 0.495	3 ± 0.54
Zinc nitrate	5±0.3	6±0.6	10 ± 0.707	5 ± 0.354
Diabetic Untreated	8±0.7	16 ± 0.3	6 ± 0.141	6 ± 0.495

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Table 8: Results of Biochemical parameters analysis

Group	D. BIL	T. PROT	ALB	GLB
Control	5 ± 0.566	54 ± 0.354	37 ± 0.707	17 ± 0.707
1mM ZnO NPs	1 ± 0.002	48 ± 0.354	38 ± 0.707	10 ± 0.354
2mM ZnO NPs	1 ± 0.03	54 ± 0.01	37 ± 0.02	17 ± 0.01
3mM ZnO NPs	3 ± 0.354	58 ± 0.707	36 ±0.636	22 ± 0.636
4mM ZnO NPs	1 ± 0.01	56 ± 0.03	37 ± 0.03	19 ± 0.02
Aqueous extract	1 ± 0.707	58 ± 0.707	35 ± 0.707	23 ± 0.283
Zinc nitrate	2 ± 0.707	60 ± 0.354	28 ± 0.707	32 ± 0.707
Diabetic untreated	3 ± 0.707	62 ± 0.707	58 ± 0.495	44 ± 0.707

Table 9: Results of Biochemical parameters analysis

Experimental Groups	N+	K+	HCO3-	CL-	UREA	CREATININE
Normal	132±.707	9.1±.2121	20±1.0607	108±1.4142	5.8±.2121	48±.2121
1mM ZnO NPs	131±1.414	5.1±.0707	24±.4243	94±.5657	9.1±.3536	71±.7071
2mM ZnO NPs	126.0±0.58	5.6±0.02	31.3±0.88	105.2±0.23	9.3±0.12	71.2±0.15
3mM ZnO NPs	133±.707	5.7±.0707	22±.6364	113±.3536	8.5±.3536	65±.2121
4mM ZnO NPs	31.0±0.58	5.8±0.02	36.3±0.88	105.2±0.15	8.6±0.09	56.2±0.12
Aqueous extract	132±1.414	5.8±.2828	23±.3536	116±.5657	12.3±.1414	100±2.8284
Zinc nitrate	133±.707	6.0±.3535	24±.3536	98±.3536	8.4±.0707	64±.7071
Diabetic Untreated	129±.707	6.9±.3535	25±.7071	90±1.4142	21.9±.4950	121±1.4142

CONCLUSION

The present study demonstrated the synthesis of ZnO NPs from different concentrations using Ficus thonningii stem bark extract via simple and stable green synthesis which acts a stabilizing and reducing agent. The characterization was achieved through UV-vis spectroscopy, FTIR and SEM analysis. However, the broad spectrum of the antibacterial activity of the biosynthesized ZnO NPs can be integrated into various medical and pharmaceutical applications for more classical treatments, effective prevention against infections

and disease-causative agents. The antidiabetic activity studied elucidated ZnO NPs as agents that can reduce blood glucose, increased insulin expression while the biochemical parametres determined as a sequestering agent on biochemical metabolism. These ZnO NPs can be used as prospective entrant for diverse of biomedical applications.

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