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ANTIOXIDANT CATALYTIC AND BIOLOGICAL ACTIVITIES OF ZINC OXIDE NANOPARTICLES SYNTHESIZED BY USING LAGERSTREOMIA SPECIO LEAVES

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ABSTRACT
Nanoparticles have surprised the mankind ever since their dawn. The uniqueness in their properties compelled the scientific community to adopt different synthetic approaches. Though each synthetic route has its own importance yet among them green synthetic method is preferred over the others because of its cost-effectiveness, eco-friendliness. We also synthesized the zinc oxide nanoparticles through green synthetic method. Zinc oxide nanoparticles prepared using Lagerstroemia Specio leaves were characterized by Ultraviolet-visible spectrophotometry, Dynamic Light Scattering, Fourier Transformer-infrared, Scanning Electron Microscopy, X-ray Diffraction for size, shape and nature. The so-prepared zinc oxide nanoparticles were studied for their various activities i.e. antioxidant activity, Catalytic activity, anti-diabetic activity, anti-microbial activity.

Keywords: Lagerstreomia leaf extract, zinc oxide nanoparticles, Uv-Visible spectrophotometry, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Fourier Transformer-Infra red (FT-IR), X-ray Diffraction (XRD), 2,2-Diphenyl picryl hydroxyl (DPPH), α-Amylase, Sodium Borohydrate.

INTRODUCTION
Nanotechnology is considered as the base of many biotechnological innovations in the 21st century and seen as the upcoming industrial revolution. Nano materials have been called 'a wonder of modern medicine' and gained much interest over the past few decades[1]. Nano materials are in possession of great potential because of their superior physicochemical and biological properties over their bulk phase. The size of nanostructured materials (1–100 nm) display a higher surface to volume ratio which led to high surface reactivity[2]. This specific property let them to be utilized in various applications in many fields ranging from material science to biotechnology. Nano biotechnology is the bridge between biotechnology and nanotechnology for developing biosynthetic and eco-friendly technology for the synthesis of nanomaterials[3]. Success in utilizing inorganic nanoparticles for biomedical applications taking into the account the environmental aspect stimulated the need to synthesize them using the green chemistry strategies via biological systems[4,5]. Various studies suggested that plants seem to be the superior candidate and are proper for large scale biosynthesis of nanoparticles where the rate of synthesis is faster than that in the case of other organisms. In addition, the nanoparticles produced through plants are more various in shape and size in comparison with those produced by other organisms such as bacteria, fungi and algae[6]. Many bioactive constituents in plants such as alkaloids, terpenoids, flavonoids, amino acids, enzymes, vitamins, proteins, and glycosides could also be a participant in bio reduction, formation and stabilization of the metal nanoparticles[7–9.] Another possible application could be its utilization in the Nano biotechnology field. Among the metal nanoparticles ZnoNPs are interesting due to its impressive properties some of which are the wide band gap, large binding energy and high piezoelectric property[10]. ZnO.NPs exhibit a wide variety of nanostructures are believed to

be bio safe, nontoxic and biocompatible, which allows them to be used in various technologies and industries such as optoelectronics, piezoelectric and magnetic sensors, bio diagnosis, biological labelling, ceramic and rubber processing, environmental protection, biology and medicinal industry[11–12].

MATERIALS AND METHOD

Materials Required: - Leaves of Lagerstreomia plant, glass beaker, filter paper, test tubes, magnetic stirrer,

Chemicals Required: - zinc sulphate, Diphenyl picryl hydroxyl (Dpph), α-amylase, sodium borohydrate, nitrophenol, congo red, methylene blue, methyl orange

METHODOLOGY

Preparation of leaf extract: - 5gm of leaf powder was taken in a glass beaker and 100ml of water was added. The mixture was boiled for 30minutes at 50˚c and then filtered. The filtrate was kept in a beaker with a label lagerstroemia leaf extract and then kept at 4˚c in refrigerator.

Synthesis of Zinc Nanoparticles: - 1ml of leaf extract was taken in glass beaker. Glass beaker was then placed on magnetic stirrer adjusted at 70c and 600rpm. Drop-wise addition of 25ml of .01M Zinc Sulphate solution was made. The mixture kept at constant conditions for 2 ½ hours turned completely into milky white colour. The mixture was then removed and kept overnight. Next day the mixture containing zinc pellets was washed by centrifuging at 13000rpm thrice to remove dirt. The Zinc pellets obtained were subjected to characterisation.

Antioxidant (Dpph Assay)

A stock solution of dpph (7mg in 10ml of methanol) was prepared. To 1ml of stock solution 10ml of methanol is added which was labeled as working solution. Different concentrations of test samples (plant extract, Zinc nanoparticles) were taken in test tubes. To each sample 800µl of methanol and 400µl of dpph was added. Dpph solution without test sample was used as control. All the samples were incubated for half an hour in dark and then absorbance was measured at 517nm.

Catalytic Assay

1ml of 0.2M freshly prepared sodium boro-hydride was taken in a cuvette and 1.9ml of 0.2Mm of dye was added to the cuvette containing sodium boro-hydride. Cuvette was shaked and placed in the uv-visible spectrophotometer to record the absorbance .The cuvette was removed and 0.1ml of test sample was added and shaken vigorously and kept in Uv-visible spectrophotometer and absorbance was recorded

Anti-Diabetic (Alpha-Amylase Assay)

20 µl of alpha amylase (.5mg/ml) were taken in test tubes. Different concentrations i.e. 15µl, 20µl, 30µl of test samples (plant extract, copper nanoparticles) and 10µl of 0.02m phosphate buffer (ph 6.9) were added to test tubes and the mixture is Incubated for 10minutes. 1ml of 1%starch solution was added to the mixture and again incubated for 20minutes. Finally 400µl of DNS reagent were added to stop the reaction and then the reaction mixture was boiled for 5minutes. Control was prepared wherein amylase is not added. Absorbance was measured at 540nm.

Antimicrobial Activity (Agar Well Diffusion Assay)

The wells, having 8 mm diameter, were punched into the nutrient agar (NA) media followed by bacterial lawn preparation in the media[13] The wells were filled with 100 µL of ZnO-NP suspension. The Petri dishes were then kept in an incubator at 37 °C for 24 h. Just after the incubation, the potency of ZnO-NPs against the tested MDR bacterial strains was determined by measuring zones of inhibition in millimetres. Wells were cut and 100 µl of the metal oxide nanoparticles were added along with the standard antibacterial agent containing disc were placed onto an agar plate. Metal salt solution was used as a standard antibacterial agent. The plates were then incubated at 37 °C for 24 hrs. The antibacterial activity was assayed by

measuring the diameter of the zone of inhibition formed around the well. The inhibition cleared zone around the sample decides the efficiency of the antibacterial agent to inhibit the growth of bacteria.

RESULTS AND DISCUSSION

Characterisation

Ultraviolet-Visible Spectrophotometry

Figure 1. shows UV-Vis absorption spectrum of zinc oxide nanoparticles. Absorption peak of the green synthesized zinc nanoparticles was observed from UV-visible spectra in the range of 360–363 nm corresponding to the characteristic peak of ZnO nanoparticles[14]. Absence of other absorbance peak in the spectra confirms that the synthesized products are pure ZnO NPs. Besides, reports are there that show the peak positions of UV-visible spectra are related with size of nanoparticles and blue shifted as the crystal size of the nanoparticles decreased[15].

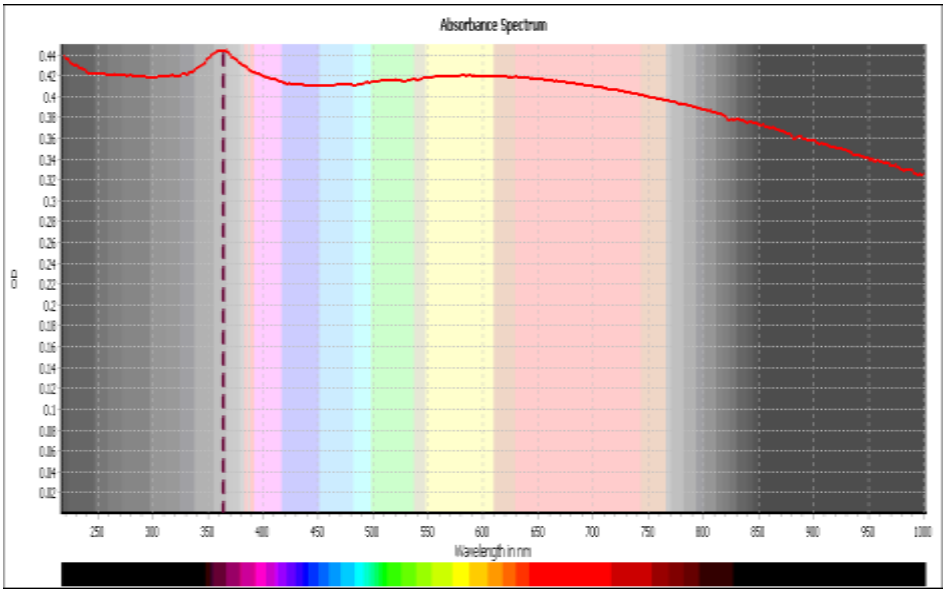


Fig. 1

Dynamic Light Scattering (DLS)

Though Debye–Scherrer's equation is used to calculate the size of nano particles of certain shape is still an approximate conviction so the trustable size measurement of NPs regardless the size and the shapes, are obtained by using either Dynamic Light Scattering (DLS) or transmission electron microscope (TEM). In the present work, the average size of the particles and size distribution of ZnO NPs was determined by Dynamic Light Scattering (DLS). According to Stokes–Einstein relation the diffusion coefficients can be converted to a hydrodynamic radius as: $D = kBT/6\pi\eta Rh$, where kB is Boltzmann's constant, T the temperature, η the viscosity of the suspension medium and Rh the hydrodynamic radius [16]. Compared to XRD, DLS is relatively rapid and inexpensive technique to measure a high number of samples. DLS provides values, which is perhaps because of the hydrodynamical shell. The size of hydrodynamical shell not only depends on the structure, but also on the shape and roughness of the particle[17].The average size of particles was recorded 38nm. Zeta potential measures the surface charge of green synthesized ZnO NPs Which is responsible for the moderate stability of the nanoparticles. The zeta potential of so-prepared ZnO NPs was measured in water as dispersion medium. The average zeta potential value recorded was −50 mV which indicates that the surfaces of biosynthesized ZnO NPs are coated with molecules which are mostly involved of negatively charged groups and likewise in charge for steadiness of the nanoparticles [18]. It can be understood that, if the value of zeta potential between 0 to ± 5, ± 10 to ± 30, ± 30 to ± 40, ± 40 to ± 60 and > ±61 mV is an indicator for rapid coagulation, incipient instability, moderate stability, good stability, and excellent stability respectively[19].

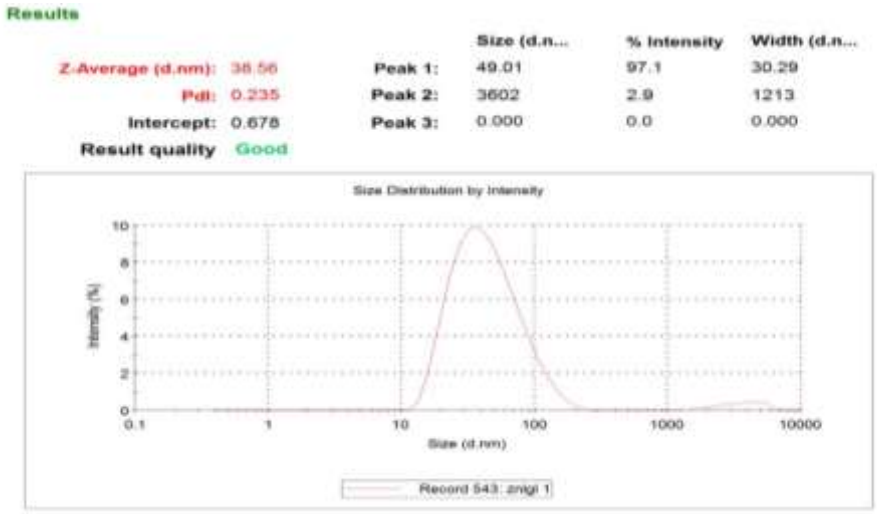


Fig. 2

Scanning Electron Microscopy (SEM)

The green synthesized zinc oxide nanoparticles were studied for morphology which was done by SEM[20]. SEM image shows that the particles are spherical in shape, and are in a highly agglomerated form, as shown in Figure 3. The particle size ranged from 32.33 to 50.02 nm, respectively. The size increase was due to the overlapping of particles on each other. We confirm the formation of ZnO-NPs by comparing the particle size obtained from X-ray diffraction and Dynamic Light Scattering . Our results are in harmony with previous reports[21].

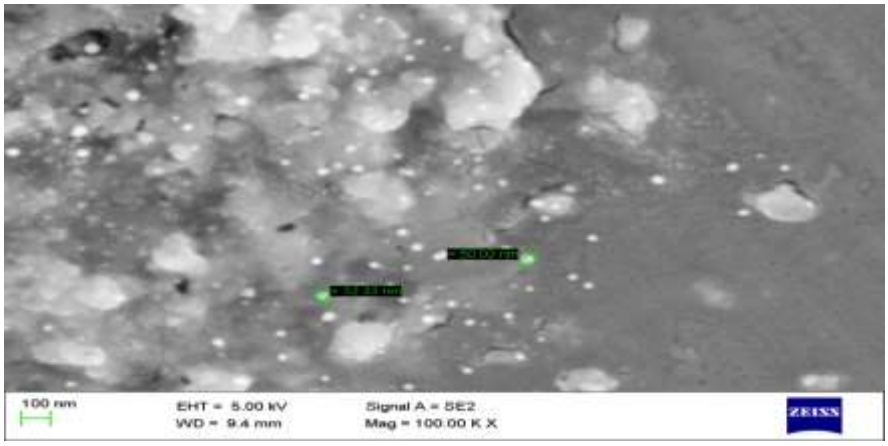


Fig. 3

Fourier Transformer-infrared (FT-IR)

The FTIR spectrum (Fig. 4) utilized to inspect the pureness and composition of biosynthesized ZnO NPs, reveals no distinct peak in the monitoring range intimating pureness of the ZnO nanoparticles produced by the green process. The peak in the region between 400 and 600 cm−1 is assigned to Zn-O stretching vibration confirming ZnO NPs are synthesized using lagerstroemia leaf extract as a reducing and capping agent. Other peaks formed at 1100 cm^{-1}, 1400 cm^{-1} , 1700 cm^{-1} 3700 cm^{-1} represent the stretches and vibrations of bonds present in the sample. As a result of the formation of ZnO nanoparticles, precisely zinc and oxygen bonding vibrations[22,23]. The band at 1363 cm−1 correlated to the C–O stretching of the carboxylic acid group. While the band found at 1483 cm−1, most probably, related to the –C=C– stretching of the aromatic compounds[24]. The robust and relatively wide band at 3600 cm−1 could be allocated to the O–H stretching of phenolic compounds[25].

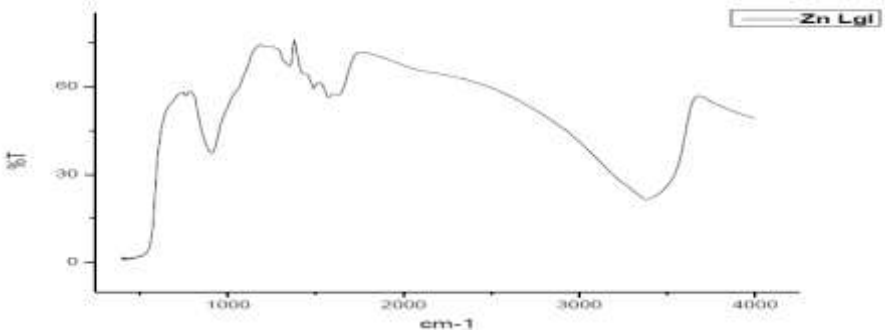


Fig. 4

X-Ray Diffraction (XRD)

X-ray diffraction analysis (Fig. 5) revealed the 2θ characteristic peaks of ZnO at 31.60°, 34.22°, 36.11°, 47.35°, 56.45°, 66.11° and 68.87°for (100), (101), (102), (103), (110), (112) and (200) planes of the crystal lattice, correspondingly. These peaks were agreeable with the regular JCPDS Card No. 89-0510 and proposed the existence of hexagonal Wurtzite form of ZnO NPs. The average crystallite sizes of ZnO NPs were calculated using Debye–Scherrer's equation, i.e. $D = k\lambda/\beta \cos\theta$, where D is crystal size, λ is the wavelength of the X-ray radiation (λ=0.15406 nm) for Cu Kα, k is shape factor typically taken as 0.89, β is the full width at high maximum (FWHM) and θ is the diffraction angle[26]. Similar outcomes were found for the biosynthesized ZnO NPs utilizing Peltophorum pterocarpum leaf extract [27] and Arabic gum [28]. The larger ZnO nanoparticles in the sample caused by the agglomeration of smaller particles, whose existence is indicated by X-ray diffraction.

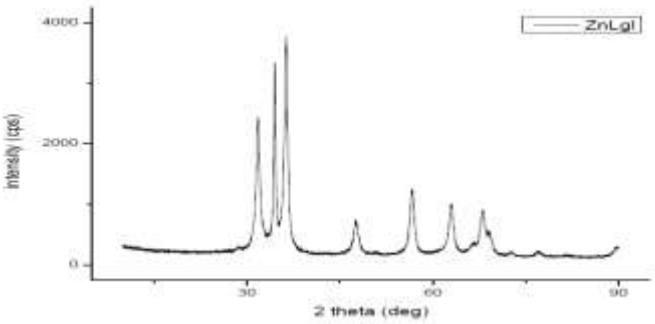


Fig. 5

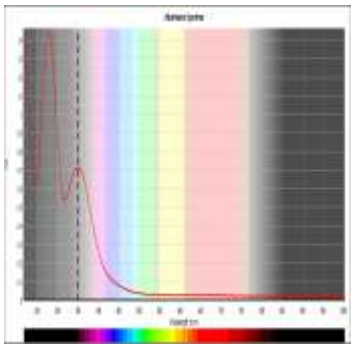
Antioxidant Activity

The change in plant metabolic pathways is attributed to environmental stress that results in reactive oxygen species (ROS) destroying membrane lipids, plant cells, DNA, and proteins[29]. Many metabolically important compounds like flavonoids, terpenoids, and oxidative stress-responsive agents play a promising role in the capping and stabilization of the nanoparticles[30, 31]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (FRSA) was conducted to assess the in vitro antioxidant potential of plant-synthesized NPs. DPPH is a stable free radical that is reduced by accepting hydrogen or electron from a donor based on formation of a yellowish diphenyl picrylhydrazine molecule[32]. The spectrophotometric method is based on quenching of stable colored radicals of DPPH, indicating the scavenging ability of the antioxidant sample. In the study, excellent free radical scavenging activity of all test concentrations was revealed, as summarized in Table 2. The highest DPPH free radical scavenging activity (70.10) and (77.71%) was recorded at 60µl for lagerstroemia leaf extract and Zinc nanoparticles respectively. The values were recorded as means of their triplicates. Our results are in harmony with previous reports[33].

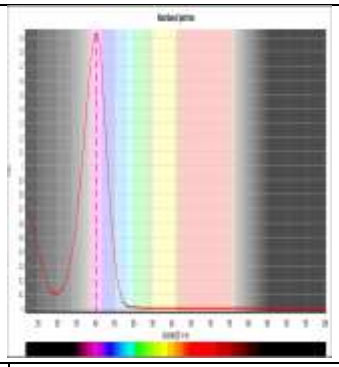
Concentration (µl)	Control (%)	Lagerstreomia leaf extract (%)	zinc-nanoparticles (%)
20	0	53.80	63.58
40	0	61.95	71.73
60	0	70.10	77.71

Catalytic Activity

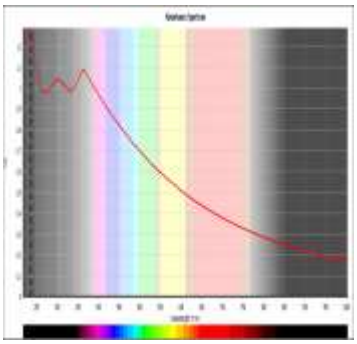
The catalytic activities of the ZnO NPs were recorded by monitoring the degradation of ortho-nitrophenol, paranitrophenol, Methylene blue and Congo red in aqueous solutions containing sodium borohydride under UV radiation. The rate of degradation was determined by recording the reduction in the absorption intensity of the organic compounds at the maximum wavelength using the UV-Vis spectrophotometer. The solutions of the organic compounds containing sodium borohydride were loaded with Zinc oxide nanoparticles and their optical absorption spectra were recorded over different durations. The colour and concentration of the organic compounds were visually observed which slowly decreased with the catalysis time. The characteristic absorption maxima of the ortho-nitrophenol, paranitrophenol, Methylene blue and Congo red at 350nm, 400nm 317nm, 400nm 600nm,665nm 500nm,330nm shifted to new absorption peaks after 30minutes of catalysis. The reduction of organic compounds by sodium borohydride is thermodynamically favourable but due to large energy barrier rate of reaction (kinetics) is slow. Hence, the addition of Zinc Methylene blue and Congo red nanoparticles increases the rate of reaction by reducing the energy barrier thus acts as catalyst. Catalytic activity of Zinc oxide nanoparticles can be attributed to the shifting of absorption peaks.



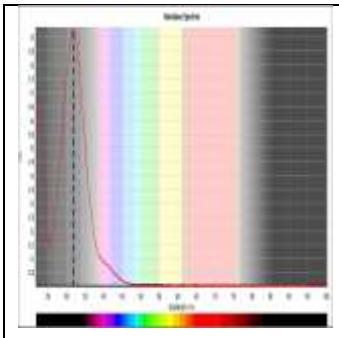
Orthonitrophenol uv-visible spectrum



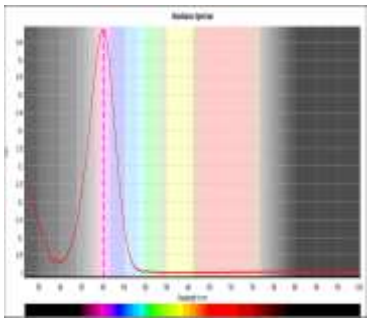
Orthonitrophenol with NABH uv-visible spectrum



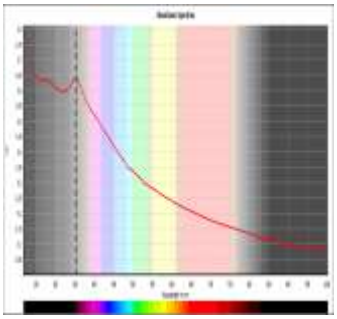
Orthonitrophenol plus NABH with zinc nanoparticles uv-visible spectrum



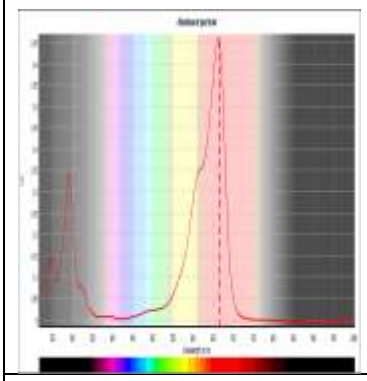
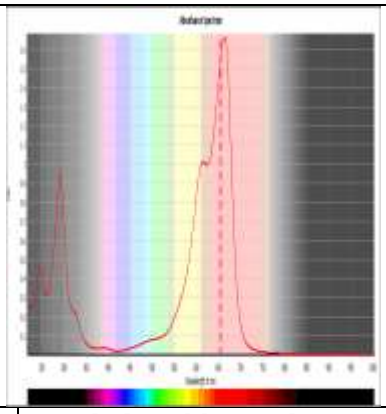
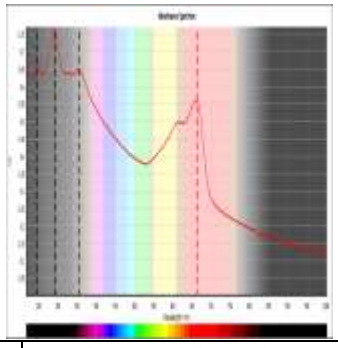
Paranitrophenol uv-visible spectrum

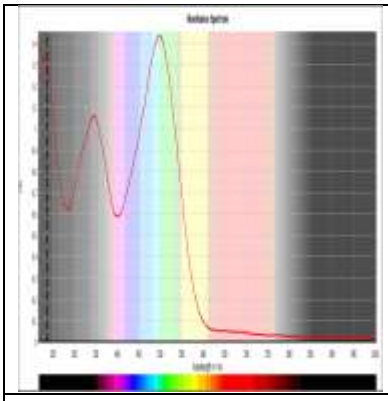
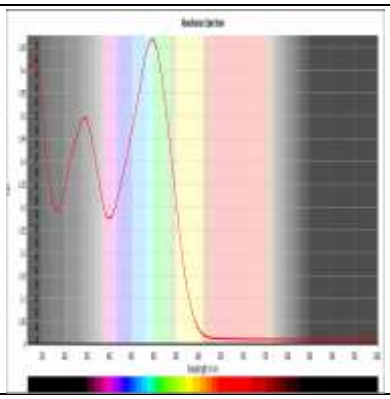
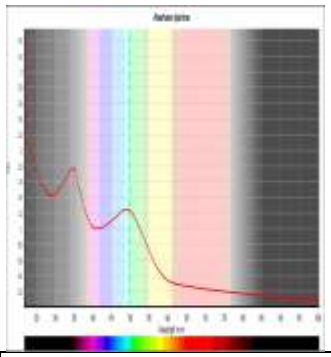


Paranitrophenol with NABH uv-visible spectrum



Paranitrophenol plus NABH with zinc nanoparticles uv-visible spectrum

		
Methylene blue uv-visible spectrum	Methylene blue with NABH uv-visible spectrum	Methylene blue plus NABH with zinc nanoparticles uv-visible spectrum

		
Congo red uv-visible spectrum	Congo red with NABH uv-visible spectrum	Congo red plus NABH with zinc nanoparticles uv-visible spectrum

Anti-Diabetic Activity

Diabetes mellitus (DM) is produced by chronic hyperglycaemia due to reduction in insulin production or inactiveness of body cells to insulin that has already been produced [34] 425 million were people with living DM according to the International Diabetes Federation (IDF) survey of 2017, and this number will increase to 629 million by 2045[35]. Effective clinical strategy for the treatment of DM is to reduce production of postprandial hyperglycaemia, which can be done by inhibiting α amylase, a carbohydrate hydrolysing enzyme in the digestive tract. Need of hour is to search for new sources of natural products with potential ant diabetic activity from tropical flora. In this study, nanoparticle samples of zinc oxide NPs were evaluated for α-amylase inhibition. Our findings indicated excellent α-amylase inhibition activity. Maximum inhibition of about 68.01 and 75.66 for αamylase was calculated at 60µl of lagerstroemia leaf extract and Zinc nanoparticles respectively. Our results are in agreement with previous reports conducted on several classes on NPs[36, 37]. From the results obtained we conclude that biosynthesized nanoparticles exhibit tremendous ant diabetic activity and are effective therapeutic agents for the treatment of diabetes.

Concentration (µl)	Control (%)	Lagerstroemia leaf extract (%)	Zinc-nanoparticles (%)
20	0	56.18	65.92
40	0	63.14	70.09
60	0	68.01	75.66

Antimicrobial Activity

Antibacterial activity of green synthesized ZnO NPs was examined against selected pathogens such as Escherichia coli, Staphylococcus aureus and Bacillus cereus by using the well diffusion method, and the result is shown in Table.The result of antibacterial activity clearly indicated that the ZnO NPs in all concentrations display a greater zone of inhibition against all bacterial strains. It may be due to the small crystal size of the Zinc nanoparticle and the sybiotic antibacterial activity of the Lagerstreomia leaf extract[38,39].Morever, the ZnO nanoparticles displayed better antibacterial activity against Gram-positive than the Gram-negative bacterial strains. Previous research report also indicated bactericidal activity of ZnO nanoparticles was greater on Gram-positive than Gram-negative bacteria [40].The reason for the difference in activity between Gram-positive and Gram-negative bacteria might be due to the differences in morphological constitutions between these microorganisms. Outer lipopolysaccharide membrane of Gram-negative bacteria makes the cell wall impermeable to antibacterial chemical substances while on other hand Gram-positive bacteria are more susceptible having only an outer peptidoglycan layer which is not permeability barrier. Hence, the cell wall of Gram-negative bacteria is more complex than the Gram-positive bacteria and making them less susceptible to the antibacterial agents [41].

Samples	**Concentration (µl)**	**Zone of inhibition (mm)**		
		Eshrichia Coli (E.coli)	Staphylococus aureus	Bacillus cereus
Control (nutrient broth)				
Zinc standard	100	12	10	11
	150	14	12	13
Lagerstreomia leaf extract	100	11	13	10
	150	13	15	12
Zinc nanoparticles	100	14	16	15
	150	17	19	18

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