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GREEN SYNTHESIS OF SILVER, ZINC AND COPPER NANOPARTICLES FROM THE AQUEOUS EXTRACT OF *TERMINALIA IVORENSIS* (*A. CHEV.*) LEAVES AND THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

In recent years, increasing antibiotic resistance by microbes is posing serious threat to humans as well as the health sector. Nanoparticles have proved to be promising candidate as antimicrobial agents, since their large surface area to volume ratio ensures a broad range of attack on bacterial surface. The current study investigated a rapid, eco-friendly and cost-effective approach to synthesis of metallic (Zn, Ag, Cu) nanoparticles (NPs) from *Terminalia ivorensis* (TI) aqueous leaf extract. The synthesized NPs were characterized by Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) analysis. Determination of the antimicrobial activities of the metallic NPs was by agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The appearance of the NPs varied from brown to dark green and were predominantly rod shaped, with the size ranging from 95-120 nm as revealed by scanning electron microscope. The UV visible spectra analysis of aqueous $AgNO_3$, $ZnSO_4$ and copper acetate showed λ max at 201, 252 and 251nm respectively., while TI extract showed λ_{max} of 660, 207 and 782 nm for Ag, Zn and Cu NPs respectively. The FT-IR study demonstrated that the aqueous plant extract acted as reducing and stabilizing agent during the synthesis of the NPs. The study revealed that all bacterial species tested showed appreciable level of susceptibility towards the green synthesized AgNP, ZnNP and CuNP. From the antibacterial susceptibility and MIC tests, the AgNP and CuNP produced better antibacterial activities than the ZnNP and the aqueous extracts.

KEYWORDS: **Antibacterial activities; Green synthesis; Nanoparticles; Spectroscopy.**

INTRODUCTION

The synthesis of nanoparticles has been the most important step in the field of nanotechnology. Nanoparticles synthesized by various chemical and physical methods have been found to be useful in producing drug delivery systems for various bioactive compounds and especially antibacterial agents.[1].The term "nanoparticles" is used to outline a particle with size in the range of 1-100 nm [2].The use of Nanoparticles for bioactive compounds of plant origin, especially those with antibacterial activity have the potential advantage of providing sustained release of the drug compounds with little or no chemical reaction and thus modulated drug degradation characteristics. It improves the solubility of poorly water-soluble antibacterial drug compounds and provides reason for better patient compliance with better therapeutic prospects [3]. Green synthesis, also referred to as biogenic synthesis, is regarded as an alternative for synthesizing metal nanoparticles. Green synthesis of

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metal nanoparticles using plant extracts is gaining importance over chemical synthesis, as it can minimize toxicity of the compound. Plant extracts with their role as surface stabilizing agents as well as reducing agent act as bio-template during the formation of nanoparticles due to, the presence of antioxidants (polyphenolic/alcoholic compounds, aldehydes/ketones) and proteins [4]. Green synthesis offers better manipulation, crystal growth control and stabilization of metal nanoparticles, and this plays a crucial role in diverse nano-technological applications [5,6]. Biosynthesized metal nanoparticles are more stable in nature and their rate of synthesis is relatively faster than conventional methods. This was demonstrated by our recent work on green synthesis of Ag, Zn and Cu nanoparticles from aqueous extract of *Spondias mombin* leaves and evaluation of their antibacterial activity [7]. Therefore, nanotechnology offers an advantage as a green route for synthesis of metal nanoparticles with plant extracts.

*Terminalia ivorensis*is is an indigenous plant from the family Combretaceae [8]. It is commonly called afàrà dúdú in Yoruba, ẹ̀ghọẹ̀n-nébì Edo and uji-oko in Igala in Nigeria, Also Emeri in Ghana and Framirein in Ivory Coast [9]. Ethno-medicinally, the pulverized leaves of the plant are used as poultice to treat burns and bruises [9]. The antibacterial activities of the ethanol extract of the plant bark against methicillin/oxacillin-resistant strains of *Staphylococcus aureus, S. epidermidis*, coagulase-negative strain has been studied [10]. This study describes a simple, faster and environment-friendly approach for synthesis of metallic (Ag, Zn, and Cu) nanoparticles using *Terminalia ivorensis* aqueous leaf extract and determination of its antimicrobial properties.

MATERIALS AND METHODS

Glassware was thoroughly washed and dried prior to use to avoid contamination. Standards of $ZnSO_4.7H_2O$, $Cu(CH_3COO)_2$ and NaOH were obtained from Sigma-Aldrich, USA. Standard stock solution of each metal ions was freshly prepared by dissolving in deionized water, while working concentrations were then prepared daily from the stock. Agilent 8453 single beam diode array UV-Visible Spectrometer, slit width of 1 nm, cuvette of 1 cm pathlength, FTIR Bruker Model and Vega 3 TESCAN SEM were used for the analysis.

Collection of plant materials

The leaves of *Termnallia ivorensis* were collected from Saki, Oyo State, Nigeria. The leaves were identified at the Botany Department, University of Lagos by Mr G.I Nodza and given a voucher specimen number LUH 7668.

Extraction

The freshly collected leaves were thoroughly washed with sterile distilled water, dried at room temperature and cut into fine pieces. The finely cut leaves (20 g) were kept in a beaker containing 100 ml deionized water and boiled for 60 min. The extract was cooled and filtered with Whatman filter paper. The extract was stored at 40^0C until further use.

Preparation of zinc nanoparticles

Zinc sulphate heptahydrate solution ($ZnSO_4.7H_2O$), 4 mM was prepared by dissolving 0.5751 g of $ZnSO_4.7H_2O$ in deionized water and the solution was transferred into a 500 ml volumetric flask and made up to mark. A 10 ml volume of the aqueous *Terminalia ivorensis* leaf extract was added to 200 ml of 4 mM $ZnSO_4.7H_2O$ solution and stirred for 5min at room temperature. After, 2 M NaOH solution was added to the mixture dropwise with continuous stirring at room temperature at pH of 12. A white precipitate resulted which was centrifuged at 10,000 rpm for 15 min. The solution was filtered using Whatman filter paper and the precipitate was dried at 60^0C for 6 h.

Preparation of silver nanoparticles

A 1 mM solution of Silver nitrate ($AgNO_3$) was prepared by weighing 0.017g of $AgNO_3$and dissolved in deionized water to one litre mark in a volumetric flask. A 5 ml volume of the *Terminalia ivorensis* leaf extract was added to 45 ml of the 1 mM $AgNO_3$ and the solution was incubated in a dark chamber. The purpose of this set-up was to prevent photo activation of silver nitrate solution at room temperature. The resultant solution was filtered using Whatman filter paper and the precipitate dried in double oven at 60 ^{0}C.

Preparation of copper nanoparticles

Copper acetate [$Cu(CH_3COO)_2$], 0.1 M solution was prepared by dissolving 9.0815 g of $Cu(CH_3COO)_2$ in deionized water and making it up in a 500ml volumetric flask. A 5 ml volume of the *Terminalia ivorensis* leaf extract was added to 45 ml of 0.1 M aqueous $Cu(CH_3COO)_2$ solution for the reduction of Cu ions. The solution was stirred for homogenous mixing until a change is observed in the colour of the solution from blue to dark green. The resultant solution was filtered using Whatman filter paper and the precipitate dried in double oven at 60 ^{0}C [7].

UV-Visible spectroscopic analysis of nanoparticles

The reduction of the pure metal ions and the formation of the nanoparticles were investigated with single beam diode array UV-Visible Spectrometer by measuring the absorbance using deionized water as blank.

Scanning electron microscopic (SEM) analysis

SEM analysis was by using Vega 3 TESCAN SEM machine (accelerated voltage of 30kV, magnification of 1.8 million times) coupled to a scandium 4.0 software. Thin films of each sample were prepared by dropping a very small amount of the sample on a carbon-coated copper grid and the film was then allowed to dry by putting it under a mercury lamp for 5 min prior to analysis.

FT-IR spectroscopic analysis of nanoparticles

Exactly 0.02g of each nanoparticle solution was dissolved in 20 mL distilled water and filtered to obtain a clear solution. Analysis was by FTIR Bruker Model with resolution ranging from 3500-500 cm^{-1} resolution.

Antimicrobial activity of the nanoparticles

The agar diffusion method was used to evaluate the antimicrobial activities of the zinc nanoparticles (ZnNP), silver nanoparticles (AgNP), copper nanoparticles (CuNP), the aqueous plant extract and ciprofloxacin as standard antibiotic drug [7]. Antimicrobial properties of the synthesized nanoparticles were investigated against the following bacterial species which are clinical isolates from our laboratory stock: *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

Standardization of inoculum of test organisms

E. coli, S. aureus and P. aeruginosa were cultured overnight in Tryptone soya broth (TSB), and then streaked onto Tryptone soya agars (TSA) which were incubated at 37^0C for 24 hrs. After 24 hours each of the organisms was taken and inoculated separately into a bottle containing 20 ml sterile normal saline and adjusted to 0.5 Mcfarland turbidity standards [7].

Preparation of samples

Concentrations of 100, 50, 25 and 12.5 % of the different nanoparticle solutions of ZnNP (0.01, 0.005, 0.0025 and 0.00125 g/ml), AgNP (0.02, 0.01, 0.005 and 0.0025 g/ml), and CuNP (0.02, 0.01, 0.005 and 0.0025 g/ml) were used as the sample solution concentrations for the antimicrobial susceptibility test. The same concentrations were used for the plant extracts. The working concentrations of ciprofloxacin used were 20, 15, 10 and 5 µg/ml.

Antimicrobial susceptibility assay by agar well diffusion

Seeded agar plates were prepared using 1 ml of each assay organism in 19 ml of Mueller Hinton agar (MHA). A cork borer of 7 mm diameter was used to bore 4 holes on each agar plate seeded with a particular organism. The wells were carefully filled with 150 uL of the different concentrations of the *Terminalia ivorensis* extract samples prepared and as labelled on the petri-dishes. The plates were allowed to rest on the bench for 4 h to allow diffusion of samples after which they were incubated at 37^oC for 24 h. Zones of inhibition around the wells were measured with a ruler after the period of incubation. This assay was done in duplicates. [7]

Determination of minimum inhibitory concentration (MIC)

Ten (10) distinct concentrations of the different samples; the plant extracts, zinc, silver and copper nanoparticle solutions were prepared as 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 %. One milliliter of each stock concentration was made up to 20 ml with molten MHA to give final concentrations of 0.984375, 1.96875, 3.9375, 7.875, 15.75, 31.5, 63, 126, 255, and 510 µg/ml in the MH agar plates. For the ciprofloxacin standard, 13 different concentration was prepared 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256, 0.512, 1.024, 2.048, 4.046 µg/ml. The mixtures were poured into petri dishes as labelled and allowed to solidify after which a drop of the various organism was carefully placed in each of the petri dishes, each plate was labelled to accommodate the three organisms, the plates were left on the bench for 4 hrs to allow diffusion to take place, after which they were incubated for 24 hrs at 37^oC. The MICs were recorded as the least concentration that produced no visible growth of the organism on the plate.

RESULTS

Complete reduction of the metals to metal ions was confirmed by the change in colour on addition of the *Terminalia ivorensis* extracts to the metal salt solution. Zinc and silver solution changed from pale yellow to brown, while copper solution changed from blue to dark green. The appearance of the NPs varied from brown to dark green and were predominantly rod shaped, with the size ranging from 95-120 nm as revealed by SEM. The UV visible spectrum for the blank and 0.1mM $AgNO_3$were as

shown in figure1 and 2 respectively. Aqueous solutions of $AgNO_3$, $ZnSO_4$ and copper acetate showed λ max at 201, 252 and 251nm respectively. The UV-Visible spectral analysis of the synthesized nano-solutions showed λ_{max} at 660,207, 782 nm for Ag, Zn, and Cu NPs respectively (Table 1). The FT-IR spectra for the aqueous plant extracts and the nanoparticles were shown in Figures 3 and 4 respectively. The FT-IR shows bond stretching at frequencies ranging from 897.64 - 3285.26, 674.95 - 3276.96and 1026.34 - 3298.64 $cm^{-1}$for the ZnNP, CuNP, and *Terminalia ivorensis* aqueous extract (TIAE) respectively (Table 2). The Zones of inhibition for synthesized AgNP, CuNP, ZnNP, and *Terminalia ivorensis* aqueous extract (TIAE) against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* revealed that the highest zones of inhibition (38 mm) was observed at 20 mg/ml for AgNP and CuNP for *E. coli*, while the zone of inhibition (40 mm) at 20 mg/ml for CuNP and (36 mm) for CuNP against *P. aeruginosa* and *S. aureus* respectively were also observed. (Tables 3). The minimum inhibitory concentration (MIC) of AgNP and CuNP of *Terminalia ivorensis* aqueous extract against *Escherichia coli*, AgNP against *Pseudomonas aeruginosa* and CuNP against *Staphylococcus aureus* to some extent corroborates the results of the zones of inhibition obtained (Table 4).

The minimum inhibitory concentration for ciprofloxacin against the test micro-organism was 0.002µg/ml for *Escherichia coli,* 0.008µg/ml for *Staphylococcus aureus,* and 1.024µg/ml for *Pseudomonas aeruginosa,* at concentrations between 0.001 – 4.046 µg/ml (Table 5).

DISCUSSION

The formation of the respective nanoparticles were marked with a colour change and the appearance of the absorbance peak at different wavelengths in the UV-Visible spectrum.The colour reaction arises from the excitation of surface plasmon vibration in the metal nanoparticles [11]. The optical properties of nanoparticles depend mainly on its surface plasmon resonance. In the nanoparticles, electron oscillates collectively which affects how light interacts with the particles and the specific oscillations depends on the particle size and shape [12].

A bathochromic shift was especially noted for the Ag and Cu NPs, this is because the energy transition was reduced in the nanoparticle resulting in higher wavelengths observed [The antimicrobial effect of the metallic nanoparticles varies with parameters including shape, size and surface charge of the nanoparticles [13]. It has been reported that the interaction between the ultra-small sized and large surface area metal nanoparticles with proteins containing sulphur in the bacterial cell wallcauses thedisruption of the cell wall which protects the interior of the cell. This results in inhibition of cell proliferation, growth, DNA damage and hence bacteria cell death [14].

The antimicrobial susceptibility test showed higher susceptibility of tested organisms to the metallic nanoparticles than to their aqueous extracts as exhibited by their wider zones of inhibition. This was more evident for the copper nanoparticles. This trend in susceptibility between the aqueous extract and the metallic nanoparticles may result from the fact that the biomolecules present in the aqueous extract of plant origin not only reduced the metal ions but also stabilized the metal nanoparticles by preventing them from being oxidized after synthesis [4]. This trend in susceptibility was corroborated by the MIC results where the metallic nanoparticle extracts gave lower MICs than the aqueous extracts. Although, the ZnNP for *T. ivorensis*, gave a higher MIC against *S. aureus* as against lower MICs exhibited by the CuNP tested. This study revealed that all bacterial species tested showed some level of susceptibility towards the green synthesized AgNP and CuNP.

CONCLUSION

A simple and rapid approach for the preparation of zinc, silver and copper nanoparticles by biological method using *Terminalia ivorensis* aqueous leaves extracts was confirmed by UV-visible spectrophotometer and FT-IR spectroscopy analysis. From this study Zn, Ag and Cu NPs showed potential as suitable antimicrobial agents against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli,* however, less active when compared to ciprofloxacin standard.

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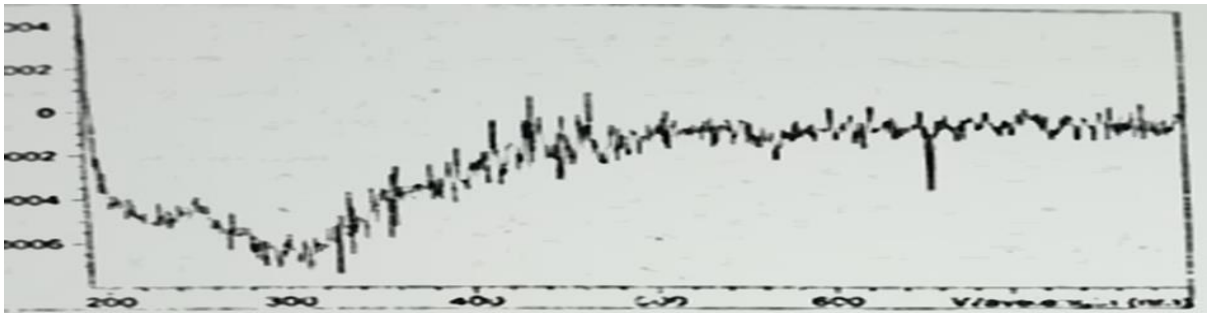

Figure 1: UV spectrophotometry blank spectrum.

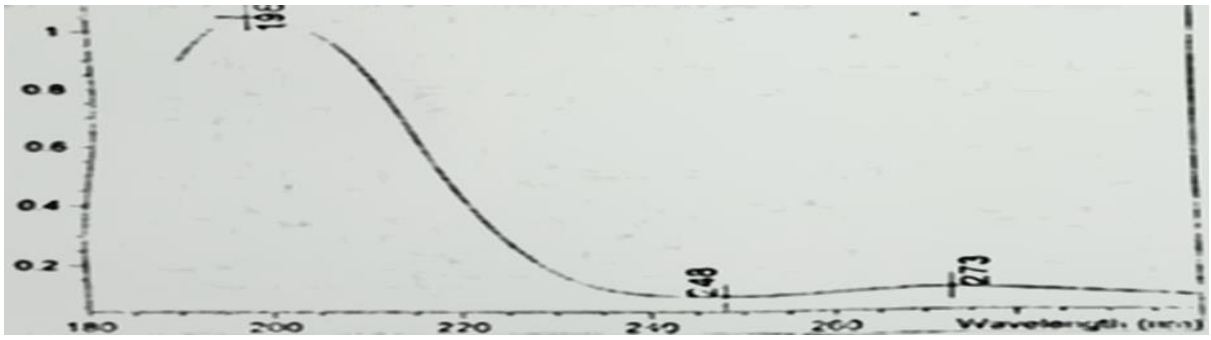

Figure 2: UV spectrophotometry spectrum of 0.1mM $AgNO_3$.

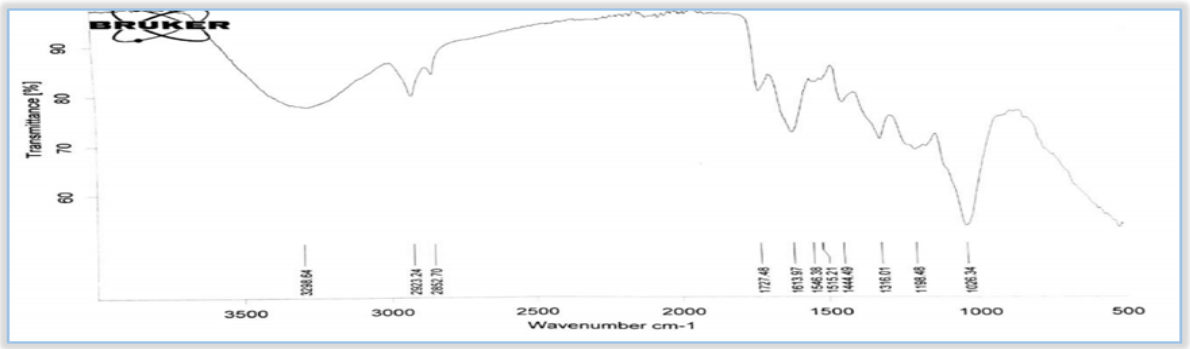

Figure 3: FTIR spectrum of *Terminalia ivorensis* aqueous extract.

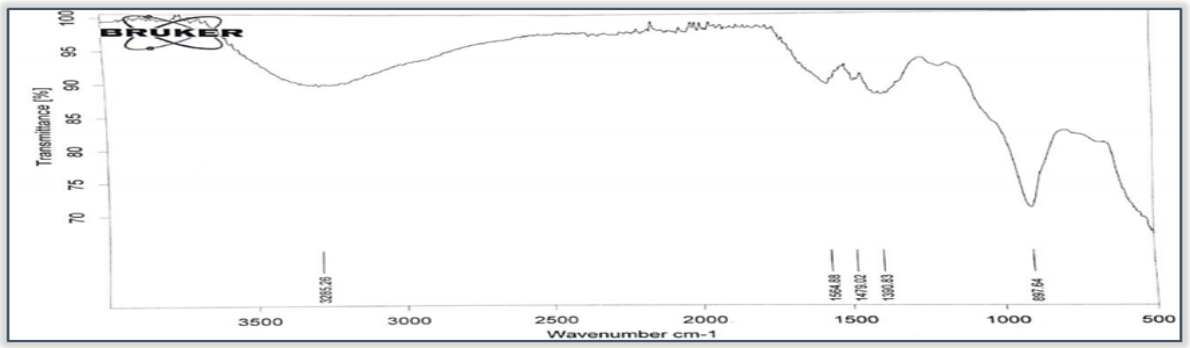

Figure 4: FTIR spectrum of *Terminalia ivorensis* zinc nanoparticles.

Table 1: UV-Visible absorbance study for the metals and synthesized AgNP, CuNP, ZnNP and *Terminalia ivorensis* aqueous extract (TIAE)

Sample	Peak (nm)	Absorbance
TIAE	585, 615, 623	0.19900, 0.18338, 0.17471
$AgNO_3$	198, 273	1.0470, 0.1121
AgNP	207, 259, 257, 357, 364	0.75191, 0.37414, 1.12260, 0.26915, 0.26915
$ZnSO_4$	252	0.0344
ZnNP	491, 574, 580, 659, 615,660	0.021519, 0.006033, 0.005518, 0.099260, 0.083303, 0.067533
$(Cu(CH_3COO)_2)$	775, 764, 770	0.20564, 0.20563, 0.20556
CuNP	774, 780, 782, 366, 600, 640	0.10749, 0.10742, 0.10727, 0.041730, 0.042313, 0.031830

Table 2: FT-IR study for synthesized CuNP, ZnNP and *Terminalia ivorensis*aqueousextract (TIAE)

TIAE		ZnNP		CuNP	
Frequency (cm^{-1})	**Bond**	**Frequency (cm^{-1})**	**Bond**	**Frequency (cm^{-1})**	**Bond**
3298.64	O-H Hydroxyl group	3285.26	O-H	3276.96	N=O nitroso
2923.24	(C-H) Alkane	1564.88	N=O nitroso	1562.86	C=C $_{STR}$
2852.70	(C-H) Alkane	1479.02	$=CH_2$ Alkene	1482.39	Sulfate
1727.48	C=O Aldehyde	1390.83	C-F	1402.01	Sulfone
1613.97	N-H of an Aromatic amine	897.64	S-OR esters	1344.61	C=S thiocarbonyl
1546.38	N =O nitroso			1195.25	C=S thiocarbonyl
1515.21	N=O nitoso			1067.55	C=S thiocarbonyl
1444.49	SO_4 Sulfate			973.28	N-O aliphatic
1316.01	C-N Cyanide			752.41	S- OR esters
1198.48	C=S thiocarbonyl			674.95	C-Cl
1026.34	C=S thiocarbonyl				

Table 3: Zone of inhibition for synthesized AgNP, CuNP, ZnNP and *Terminalia ivorensis*aqueous extract (TIAE) against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*

	Zone of inhibition (mm)											
Conc.		*Escherichia coli*				*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
(mg/ml)	TIAE	AgNP	ZnNP	CuNP	TIAE	AgNP	ZnNP	CuNP	TIAE	AgNP	ZnNP	CuNP
20	30	38	36	38	28	30	-	40	28	28	34	36
10	28	30	-	28	-	28	-	36	26	36	24	2 6
5	26	-	-	18	-	-	-	-	-	30	-	24
2.5	20	-	-	-	12	-	-	-	-	20	-	-

(-) no zone of inhibition; *Terminalia ivorensis*aqueous extract (TIAE), NP = nanoparticle; Ag = silver; Zn = zinc; Cu = copper

Table 4: Minimum inhibitory concentration of *Terminalia ivorensis*aqueousextract (TIAE), AgNP, ZnNP and CuNP against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

	Minimum inhibitory concentration											
Conc	*Escherichia coli*				*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
(µg/ml)	TIAE	AgNP	ZnNP	CuNP	TIAE	AgNP	ZnNP	CuNP	TIAE	AgNP	ZnNP	CuNP
0.98	+	+	+	+	+	+	+	+	+	+	+	+
1.97	+	+	+	+	+	+	+	+	+	+	+	+
3.94	+	+	+	+	+	+	+	+	+	+	+	+
7.88	+	-	+	+	+	+	+	+	+	+	+	+
15.75	+	-	+	-	+	-	+	+	+	+	+	+
31.5	-	-	+	-	+	-	+	+	+	+	+	-
63	-	-	+	-	+	-	+	-	+	-	+	-
126	-	-	-	-	-	-	+	-	-	-	-	-
255	-	-	-	-	-	-	-	-	-	-	-	-
510	-	-	-	-	-	-	-	-	-	-	-	-
MIC (µg/ml)	31.5	7.88	126	15.75	126	15.75	255	63	126	63	126	32

(+) growth of organism, (-) no growth of organism; *Terminalia ivorensis* aqueous extract (TIAE); NP= nanoparticle; Ag = silver; Zn = zinc; Cu = copper

Table 5: Minimum inhibitory concentration of ciprofloxacin standard *against Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Concentration (µg/ml)	*E. coli*	*S. aureus*	*P. aureginosa*
0.001	+	+	+
0.002	-	+	+
0.004	-	+	+
0.008	-	-	+
0.016	-	-	+
0.032	-	-	+
0.064	-	-	+
0.128	-	-	+
0.256	-	-	+
0.512	-	-	+
1.024	-	-	-
2.048	-	-	-
4.046	-	-	-
MIC (µg/ml)	0.002	0.008	1.024

(+) growth of organism, (-) no growth of organism.

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