

Adeyemi et al. Afr. J. Clin. Exper. Microbiol. 2020; 21 (2): 106 - 113

<https://www.afrcem.org>African Journal of Clinical and Experimental Microbiology ISSN 1595-689X
AJCEM/1970: <https://www.ajol.info/index.php/ajcem>

Apr 2020 Vol.21 No.2

Copyright AJCEM 2020: <https://dx.doi.org/10.4314/ajcem.v21i2.4>**Original Article****Open Access****Green synthesis of Ag, Zn and Cu nanoparticles from aqueous extract of *Spondias mombin* leaves and evaluation of their antibacterial activity**^{*1}Adeyemi, D. K., ²Adeluola, A. O., ¹Akinbile, M. J., ¹Johnson, O. O., and ¹Ayoola, G. A.¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria²Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria*Correspondence to: dadeyemi@unilag.edu.ng; dkadeyemi@yahoo.com; +2348033871465**Abstract:**

Background: Nanotechnology offers an advantage as a green route for synthesis of metal nanoparticles (NPs) with plant extracts as capping agent. *Spondias mombin* is a fruit-bearing tree and its leaf extracts have been reported to possess anxiolytic, hypoglycaemic, antiepileptic, antipsychotic, sedative, antioxidant, and antimicrobial properties. The objective of the study is to determine the antibacterial potential of a simple non-toxic product of green synthesis of metallic (Ag, Zn and Cu) nanoparticles using the leaf of *Spondias mombin* aqueous extracts (SMAE) as a reducing and capping agents of the metal ions.

Methodology: Nanoparticles were characterized by UV visible spectrophotometric analysis, Fourier Transform Infra-Red (FT-IR) spectrophotometer and scanning electron microscope (SEM). Antimicrobial activities of synthesized NPs against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were determined by agar well diffusion technique.

Results: The synthesized NPs varied in colour from dark brown to green and appears dominantly spherical, occasionally rod or triangular shaped with size ranging from 65-90 nm. UV spectroscopy absorption spectra of Ag, Zn and Cu NPs had absorbance peak at 267, 262 and 765 nm respectively. FT-IR spectrometry of Zn NP, Cu NP, and SMAE gave wave number ranging from 895.71-3320.67, 747.02-3225.45 and 658.25-3674.49 respectively. FT-IR analysis showed that SMAE acted as reducing and stabilizing agent while the NPs exhibited lower energy absorption band when compared to the plant extract. The NPs demonstrated higher antimicrobial activities against *S. aureus* than *Ps. aeruginosa* and *E. coli*. The antimicrobial activity was higher with copper NP than Ag and Zn NPs, and also higher than SMAE.

Conclusion: The result from this study presents an indication for an alternative means for development of novel antimicrobial agents for clinical and biotechnological applications.

Keywords: synthesis, nanoparticles, FT-IR, UV-visible spectrophotometry, antibacterial activities

Received July 3, 2019; Revised October 24, 2019; Accepted October 26, 2019

Copyright 2020 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source.**Synthèse verte de nanoparticules d'Ag, de Zn et de Cu à partir d'extrait aqueux de feuilles de *Spondias mombin* et évaluation de leur activité antibactérienne**^{*1}Adeyemi, D. K., ²Adeluola, A. O., ¹Akinbile, M. J., ¹Johnson, O. O., and ¹Ayoola, G. A.¹Département de chimie pharmaceutique, Faculté de pharmacie, Université de Lagos, Nigéria²Département de pharmacie et de technologie pharmaceutique, faculté de pharmacie, université de Lagos, Nigeria*Correspondance à: dadeyemi@unilag.edu.ng; dkadeyemi@yahoo.com; +2348033871465

Abstrait:

Contexte: La nanotechnologie offre un avantage en tant que voie verte pour la synthèse de nanoparticules métalliques (NP) avec des extraits de plantes comme agent de coiffage. *Spondias mombin* est un arbre fruitier et ses extraits de feuilles possèdent des propriétés anxiolytiques, hypoglycémiques, antiépileptiques, antipsychotiques, sédatives, antioxydantes et antimicrobiennes. L'objectif de l'étude est de déterminer le potentiel antibactérien d'un simple produit non toxique de synthèse verte de nanoparticules métalliques (Ag, Zn et Cu) à l'aide de la feuille d'extraits aqueux de *Spondias mombin* (SMAE) en tant qu'agent réducteur et coiffant de la ions métalliques.

Méthodologie: Les nanoparticules ont été caractérisées par analyse spectrophotométrique UV visible, spectrophotomètre à transformée de Fourier infrarouge (FT-IR) et microscope électronique à balayage (MEB). Les activités antimicrobiennes de NP synthétisées contre *Escherichia coli*, *Staphylococcus aureus* et *Pseudomonas aeruginosa* ont été déterminées par une technique de diffusion sur puits d'agar.

Résultats: Les NP synthétisés ont une couleur allant du brun foncé au vert et apparaissent principalement sphériques, parfois en bâtonnets ou en triangles, avec des tailles allant de 65 à 90 nm. Les spectres d'absorption par spectroscopie UV des NP Ag, Zn et Cu présentaient des pics d'absorbance à 267, 262 et 765 nm respectivement. La spectrométrie FT-IR de Zn NP, Cu NP et SMAE a donné un nombre d'onde allant de 895,71 à 3320,67, 747,02 à 3225,45 et 658,25 à 3674,49 respectivement. L'analyse FT-IR a montré que le SMAE agissait en tant qu'agent réducteur et stabilisant, alors que les NP présentaient une bande d'absorption d'énergie inférieure à celle de l'extrait de plante. Les NP ont démontré des activités antimicrobiennes plus élevées contre *S. aureus* que *Ps. aeruginosa* et *E. coli*. L'activité antimicrobienne était plus élevée avec les NP en cuivre que dans les NP Ag et Zn, et également supérieure à celle du SMAE.

Conclusion: le résultat de cette étude présente une indication d'un autre moyen de développement de nouveaux agents antimicrobiens pour des applications cliniques et biotechnologiques.

Mots-clés: synthèse, nanoparticules, FT-IR, spectrophotométrie UV-visible, activités antibactériennes

Introduction:

Nanotechnology is unfolding as a highly advanced multidisciplinary technology involving chemistry, physics, material science, biology and medicine. The synthesis of nanoparticles (NPs) has been the most important stride in the area of nanotechnology (1). In the subject of biology, NPs have diverse use in drug delivery systems, antibacterials, minerals and many others. There are wide spans of chemical and physical methods being utilized for the synthesis of NPs. Nonetheless, these methods have few limitations such as the use of dangerous solvents, high energy utilization, and dangerous byproducts.

The use of plant extracts in the green synthesis of NPs is attaining significance over chemical synthesis. Plant extracts (possessing intrinsic ability) as surface stabilizing agents, may act as bio-template for the synthesis of NPs with potential for better crystal growth control and stabilization. This class of NPs synthesis plays a vital role in diverse nanotechnological applications (2, 3).

Spondias mombin is a fruit-bearing tree which belongs to the genus *Spondias*, a flowering plant family, Anacardiaceae, home to the lowland slightly wet forest of the Amazon (4, 5). The dispersal of this plant reach all of tropical America, Brazil, Nigeria, West Indies and other tropical rain forests worldwide (5, 6). *S. mombin* enamate gum is used as adhesives, its bark and root decoctions are

used as purgatives and medicament for dysentery, diarrhea, and haemorrhoids, while leaf extracts of the tree have been reported to possess anxiolytic, antiépileptic, anathematic, sedative, antipsychotic, antioxidant, and antimicrobial properties (7-11).

The biomolecules existing in plants can act as capping and reducing agents and thus increase the rate of reduction and stabilization of NPs. Biosynthesized metal NPs are more stable in nature and their synthesis rate is faster than other procedures. Some degree of antibacterial and antifungal activities have been demonstrated in green synthesized NPs of zinc and silver oxide of aqueous extract of some plants (12, 13, 14). In this study, the antibacterial activity of *Spondias mombin* leaf extract synthesized silver (Ag), zinc (Zn) and copper (Cu) NPs was determined against our laboratory stocks of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical isolates.

Materials and methods:

Collection and preparation of plant materials

Spondias mombin leaf was collected from Ijegun, Lagos State, Nigeria. Identification was done at the Botany department, University of Lagos, Akoka, Lagos with voucher number LUH 7667. Healthy leaves were hand-picked and isolated from the rest. The freshly collected leaf was carefully washed with distilled water, dried at room temperature and

cut into fine pieces. 20g of finely cut leaf were kept in a separate beaker containing 100 ml deionized water (0.2g/ml) and boiled for 60 mins. The extract was cooled and filtered with Whatman filter paper and stored at 40°C till further use.

Preparations of Ag, Zn and Cu nanoparticles

Preparations of silver (Ag) NP

A 1mM of AgNO₃ was prepared by weighing 0.017g of AgNO₃ and dissolved in deionized water. The solution was then transferred into a volumetric flask and filled up to 1 liter mark. Five milliliter (1g) of the *Spondias mombin* leaf extract was added to 45ml (0.02g/ml) of 1mM AgNO₃. The composition was incubated in a dark chamber to decrease photo-activation of AgNO₃ at room temperature.

Preparations of zinc (Zn) NP

A 4mM solution of zinc sulphate heptahydrate solution (ZnSO₄.7H₂O) was prepared by dissolving 0.5751g of ZnSO₄.7H₂O in deionized water. The solution was transferred into a volumetric flask and filled up to 500 ml mark. Ten milliliter (2g) of the aqueous *Spondias mombin* leaf extract was added to 200ml of 4mM ZnSO₄.7H₂O solution (0.01g/ml) and stirred for 5 minutes at room temperature. Then, 2M NaOH was added to the mixture dropwise with constant stirring at room temperature, and pH sustained at 12. The resulting white precipitate was centrifuged at 10,000rpm for 15mins. The solution was filtered with Whatman filter paper and washed twice with sterile distilled water, after which the precipitate was dried at 60°C for 6 hrs.

Preparation of copper (Cu) NP

A 0.1M copper acetate [Cu(CH₃COO)₂] solution was prepared by dissolving 9.0815g of Cu(CH₃COO)₂ in deionized water and making it up in a 500ml volumetric flask. Five milliliter (1g) of *Spondias mombin* leaf extract was added to 45ml of 0.1M aqueous Cu(CH₃COO)₂ solution (0.02g/ml) for the reduction of Cu²⁺ ions. The solution was stirred for homogenous mixing, a change was observed in the colour solution from blue to dark green. The resultant solution was filtered with Whatman filter paper and the precipitate was dried in the oven at 60°C.

Characterization of synthesized nanoparticles

Scanning electron microscope (SEM)

SEM analysis was done by using Vega 3 Tescan SEM machine coupled to Scandium

4.0 software. Thin films of Ag, Cu, and Zn NPs of SMAE each sample was 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.

UV-visible spectroscopy analysis of NPs

The reduction of the pure metal ions and the formation of the nanoparticles were ascertained using double beam UV-visible spectrometer (PG Instruments Ltd, T80 +, S/N 15-1885-01-0094) to measure the absorbance of solution at a resolution of 1 nm from 180 to 900 nm, using deionized water as blank.

FT-IR spectroscopy analysis of NPs

Approximately 0.02g of NP solutions was dissolved in 20ml distilled water and filtered to obtain a clear solution. The FTIR spectra were then recorded with detector at 4000-400 cm⁻¹ resolution and 20 scans per sample.

Antimicrobial activities of synthesized NPs

Antimicrobial activities of the synthesized NPs were investigated against clinical isolates of *E. coli*, *S. aureus* and *Ps. aeruginosa* using agar well diffusion to determine the antimicrobial activity, and agar dilution to determine the MICs of each of the synthesized NPs in comparison with aqueous plant extract and ciprofloxacin as control.

Standardization of inoculum of test organisms

The overnight cultures of *E. coli*, *S. aureus* and *Ps. aeruginosa* in Tryptone soya broth (TSB) were streaked onto Tryptone soya agar (TSA) which were incubated at 37°C for 24 hours. Colonies of the organisms were emulsified in a bottle containing 20ml sterile normal saline, and their turbidity was adjusted to match that of 0.5 McFarland standards.

Preparation of samples

A 100%, 50%, 25% and 12.5% concentrations of the different NP solutions of Ag (0.02 g/ml, 0.01 g/ml, 0.005 g/ml, 0.0025 g/ml), Zn (0.01 g/ml, 0.005 g/ml, 0.0025 g/ml, 0.00125 g/ml) and Cu (0.02 g/ml, 0.01 g/ml, 0.005 g/ml, 0.0025 g/ml) and similar concentrations for the plant extract were used for the assay. The MIC working concentrations of ciprofloxacin used were 20µg/ml, 15µg/ml, 10µg/ml and 5µg/ml.

Antibiotic susceptibility test by agar diffusion

Different sections of Mueller Hinton (MH) agar plates used were assigned to the various concentrations of *Spondias mombin*

extract and the synthesized Ag, Cu, and Zn NPs. 1 ml of the standardized inoculum of each organism was pipetted onto the surface of its assigned MH agar plates and allowed to spread on the surface. A sterile cork borer of size 7mm was used to create 4 wells on each plate. The wells were filled with 150 μ L of the test and control samples as assigned on each plate. The plates were left on the bench for 4 hrs to allow for diffusion of samples, after which they were incubated for 24 hrs at 37°C. The zones of inhibition were measured with a calibrated ruler. The assay for each sample was done in duplicates.

Determination of minimum inhibitory concentration (MIC) by agar dilution

Ten distinct stock concentrations of the different samples of the plant extract, and Ag, Zn and Cu NP solutions were prepared as 0.1% (0.0196875 mg/ml), 0.2% (0.039375 mg/ml), 0.4% (0.07875mg/ml), 0.8% (0.1575 mg/ml), 1.6% (0.315 mg/ml), 3.2% (0.630 mg/ml), 6.4% (1.260.0 mg/ml), 12.8% (2.52 mg/ml), 25.6% (5.1 mg/ml) and 51.2% (10.2 mg/ml). One milliliter of each stock concentration was made up to 20 ml on molten MH agar to give final concentrations of 0.984375 μ g/ml, 1.96875 μ g/ml, 3.9375 μ g/ml, 7.875 μ g/ml, 15.75 μ g/ml, 31.5 μ g/ml, 63 μ g/ml, 126 μ g/ml, 255 μ g/ml, and 510 μ g/ml in the MH agar plates. For ciprofloxacin, 13 different concentrations were prepared; 0.001 μ g/ ml, 0.002 μ g/ml, 0.004 μ g/ml, 0.008 μ g/ml, 0.016 μ g/ml, 0.032 μ g/ml, 0.064 μ g/ml, 0.128 μ g/ml, 0.256 μ g/ml, 0.512 μ g/ml, 1.024 μ g/ml, 2.048 μ g/ml

Approximately 0.1 ml of standardized inoculum of each organism was plated on the MH agar containing respective concentrations of the extract, synthesized NPs and ciprofloxacin. The plates were left on the bench for 4 hours to allow diffusion of the inoculum into the agar, after which they were incubated for 24 hours at 37°C. The MICs were recorded as

the least concentration that produced no growth of the organism on the plate.

Results:

As shown by the SEM, the NPs varied in colour from dark brown to green and appears dominantly spherical, occasionally rod or triangular shaped with the size ranging from 65-90 nm. Complete reduction of the metals to metal ions was confirmed by the colour change on addition of the *Spondias mombin* extract to the metal salt solution. Silver and zinc solution changed from pale yellow to brown while copper solution changed from blue to dark green. UV-visible spectroscopy absorption spectra of Ag, Zn and Cu NPs had an absorbance peak at 267, 262 and 765 nm (Table 1).

The Fourier-Transform Infrared (FT-IR) spectroscopy for the synthesized Zn NP, Cu NP, and *Spondias mombin* aqueous extract (SMAE) showed wave number ranging from 895.71-3320.67, 747.02-3225.45, and 658.25-3674.49 respectively (Table 2).

The zones of inhibition for *Spondias mombin* aqueous extract and synthesized Ag, Cu, and Zn NPs against *E. coli*, *S. aureus* and *Ps. aeruginosa* are shown in Table 3. The highest zone of inhibition was observed at 20 mg/ml with SMAE zone of inhibition of 26 mm against *E. coli*, and Cu NP of 40 mm and 36 mm against *Ps. aeruginosa* and *S. aureus* respectively.

The MICs of *Spondias mombin* aqueous extract and the synthesized Ag, Zn and Cu NPs against *E. coli*, *Ps. aeruginosa* and *S. aureus* are shown in Table 4. The MICs of ciprofloxacin are 0.002 μ g/ml for *E. coli*, 0.008 μ g/ml for *S. aureus* and 1.024 μ g/ml for *Ps. aeruginosa* (Table 5). Antimicrobial effect was observed to be higher in Gram positive bacteria (*S. aureus*) than Gram negative bacteria (*E. coli* and *Ps. aeruginosa*) and most especially for Cu NP.

Table 1: UV-visible absorbance of Ag NP, Cu NP, Zn NP and SMAE

Sample	Peak (nm)	Absorbance
SMAE	490, 655, 581, 267, 363, 405	0.1822, 0.1765, 0.1503, 1.1113, 0.2923, 0.1290
AgNO ₃	198, 273	1.0470, 0.1121
Ag NP	489, 598, 647, 267, 922, 655, 546, 429, 463, 438, 579	0.0163, 0.0241, 0.0142, 0.7306, 0.1507, 0.1560, 0.0071, 0.0149, 0.0818, 0.0447, 0.0209
ZnSO ₄	252	0.0344
Zn NP	262, 489, 590, 358, 372	0.6209, 0.6372, 0.3664, 0.2269, 0.0886
[Cu(CH ₃ COO) ₂]	764, 743, 780, 797, 819, 848	0.2056, 0.0330, 0.0165, 0.0333, 0.0154, 0.0148
Cu NP	765,490,585, 598, 427, 358,397, 366, 372	0.8897, 0.6841, 0.3823, 0.3671, 0.4620, 0.3366, 0.2158, 0.0779, 0.0651

NP = nanoparticle; Ag = silver; Cu = copper; Zn = zinc; SAME = *Spondias mombin* aqueous extract

Table 2: Fourier Transform Infra-Red (FT-IR) spectrophotometry of synthesized Cu NP, Zn NP and SMAE

SMAE		Zn NP		Cu NP	
Wave no (cm ⁻¹)	Functional group	Wave no (cm ⁻¹)	Functional group	Wave no (cm ⁻¹)	Functional group
3674.49, 3278.52	OH of alcoholic and phenolic group	3320.67	OH of alcoholic and phenolic group	3225.45	OH of alcoholic and phenolic group
2919.75, 2851.23	C-H stretching vibration of an alkyl group	1581.52	N-H band of primary amines	1708.33	C=O carbonyl stretching vibration
1976.79	C=C=C	1493.16	N-H stretching of aromatic amino group	1692.12	C=C stretching vibration of an aromatic or alkene
1726.44	C=O carbonyl stretching vibration	1481.91	N-H stretching of aromatic amino group	1582.84	N-H band of primary amines
1602.88	C=C stretching vibration of an aromatic or alkene	1467.88	N-H stretching of aromatic amino group	1479.44, 1440.84	N-H stretching of aromatic amino group
1439.52, 1372.49-1323.19	N-H stretching of aromatic amino group	895.71	C-H deformed of alkenes	1343.13	O-H bending vibration
1222.13-1160.03	C-O stretching vibration)			1179.98	C-C
1030.72	C-N bending vibration			1033.22	C-N bending vibration
817.34, 774.41-717.34	C-H deformed of alkenes			966.55	
658.25	Alkanes			747.02	Alkanes

Table 3: Zones of inhibition for synthesized Ag NP, Cu NP, Zn NP and SMAE against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

Conc. mg/ml	Zones of inhibition											
	<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>					<i>Staphylococcus aureus</i>			
	SMAE	Ag NP	Zn NP	Cu NP	SMAE	Ag NP	Zn NP	Cu NP	SMAE	Ag NP	Zn NP	Cu NP
20	26mm	16mm	ND	16mm	24mm	32mm	ND	40mm		20mm	ND	36mm
10	20mm	10mm	16mm	12mm	20mm	-	14mm	38mm		16mm	26mm	30mm
5	20mm	-	12mm	-	20mm	-	10mm	16mm		12mm	24mm	24mm
2.5	12mm	-	-	-	12mm	-	10mm	14mm		10mm	-	14mm
1.25	ND	ND	-	ND	ND	ND	-	ND		ND	-	ND

ND = not done; (-) = no zone of inhibition; SMAE - *Spondias mombin* aqueous extract; NP = nanoparticle; Ag = silver; Zn = zinc; Cu = copper

Table 4: Minimum inhibitory concentration of SMAE, Ag NP, Zn NP and Cu NP against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Conc µg/ml	Minimum inhibitory concentration											
	<i>Escherichia coli</i>				<i>Pseudomonas aeruginosa</i>				<i>Staphylococcus aureus</i>			
	SMAE	Ag NP	Zn NP	Cu NP	SMAE	Ag NP	Zn NP	Cu NP	SMAE	Ag NP	Zn NP	Cu NP
0.98	+	+	+	+	+	+	+	+	+	+	+	+
1.97	+	+	+	+	+	+	+	+	+	+	+	+
3.94	+	+	+	+	+	+	+	+	+	+	-	+
7.88	+	+	+	+	+	+	+	+	+	+	-	+
15.75	+	+	+	+	+	+	+	+	+	+	-	-
31.5	+	+	+	-	+	+	-	-	+	-	-	-
63	+	+	+	-	+	+	-	-	-	-	-	-
126	+	+	+	-	-	+	-	-	-	-	-	-
255	+	+	+	-	-	+	-	-	-	-	-	-
510	-	-	-	-	-	-	-	-	-	-	-	-
MIC µg/ml	510	510	510	31.5	126	510	31.5	31.5	63	31.5	3.94	15.75

(+) = growth of organism; (-) = no growth of organism; SMAE = *Spondias mombin* aqueous extract; NP= nanoparticle; Ag = silver; Zn = zinc; Cu = copper

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

Concentration (µg/ml)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
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	0.002 µg/ml	0.008 µg/ml	1.024 µg/ml

(+) = growth of organism; (-) = no growth of organism

Discussion:

Spondias mombin is a fruit-bearing tree. The leaf extracts have been reported to possess anxiolytic, hypoglycaemic, antiepileptic, antipsychotic, sedative, antioxidant, and antimicrobial properties (7-11). In this study, green synthesized metallic NPs were identified by UV-visible and Fourier Transform Infra-Red spectrophotometer. While the generation and steadiness of the reduced metal NPs in the colloidal solution were scanned by UV-visible spectrophotometer, the FT-IR showed the

feasible biomolecules for the reduction of metallic NPs.

The NPs varied in colour from dark brown to green color and were rod shaped, with the size ranging from 65-90 nm as revealed by the SEM. The appearance of colour change from pale yellow to brown for Ag and Zn solution, and from blue to dark green for Cu showed the formation of the respective NPs. Metal NPs such as Ag, Zn and Cu have free electrons, which give rise to surface plasmon resonance absorption band. The excitation of surface plasmon vibration in the metal NPs

gave rise to the colour reaction (15, 16). Subhankari and Nayak (17) have also reported that the biomolecules confirmed to be present in the aqueous extract of plant origin reduced the metal ions in addition, and also stabilized the metal NPs by blocking them from being oxidized after synthesis.

The zones of inhibition exhibited with *Spondias mombin* aqueous extract synthesized Cu NP in this study agrees with those of Pragati et al., (12), Palaniselvam et al., (13), Logeswari et al., (14), and Thatoi et al., (18). The Cu NP gave the highest antimicrobial activities against *Ps. aeruginosa* and *S. aureus* followed by Ag NP against *Ps. aeruginosa*. The SMAE alone had higher activity than Ag and Zn NPs. Although the ciprofloxacin standard gave much lower MIC values which were also well within the 2016 CLSI breakpoints of $\leq 1 \mu\text{g/ml}$ for susceptibility to ciprofloxacin (19) for the isolates used, these cannot be compared with the SMAE extract and synthesized NPs. This is because the actual compounds in the extract responsible for the antimicrobial activity were not determined in our study.

Metallic NPs are being reported to have desired antibacterial activity because of their large surface area to volume ratio (20). It is quite noteworthy that all bacterial species tested in this study showed appreciable level of susceptibility towards the green synthesized NPs.

Conclusion:

A simple development of Ag, Zn and Cu NPs by biological method using *Spondias mombin* aqueous leaf extracts was established by UV-visible spectrophotometer and FT-IR spectroscopy analysis. From this study, synthesized Ag, Zn and Cu NPs were found to possess antibacterial potential against *E. coli*, *S. aureus*, and *Ps. aeruginosa* though with comparatively less activity than ciprofloxacin that was used as standard.

Acknowledgements:

The authors gratefully acknowledge the Director, Central Research Laboratory, University of Lagos, for permission to use the laboratory facilities. The authors appreciate Mr. G. I. Nodza of the Department of Botany, University of Lagos, for assisting in the plant identification.

References:

- Albrecht, M. A., Evans, C. W., and Raston, C. L. Green chemistry and the health implications of nanoparticles. *Green Chem.* 2006; 8: 417-432
- Juhi, S., Madan, M. S., Sarika, G., and Abhijeet, S. Emerging role of fungi in nanoparticle synthesis and their applications. *World J Pharm Sci.* 2014; 3: 1586-1613
- Monalisa P., and Nayak, P. L. Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract. *Int J Plant, Animal Environ Sci.* 2013; 3: 68-78.
- Adedokun, M. O., Oladoye, A. O., Oluwalana, S. A., et al. Socio-economic importance and utilization of *Spondias mombin* in Nigeria. *Asian Pac J Trop Med.* 2010; 3: 232-234
- Bicas, J., Molina, G., Dioniso, P. A., Barros, F. F., et al. Volatile constituents of exotic fruits from Brazil. *Food Res Int.* 2011; 44: 1843-1855
- Mattietto, R. A., and Matta, V. Cajá (*Spondias mombin* L.). In Yahia, E. (ed). *Postharvest Biology and Technology of Tropical and Subtropical Fruits.* Cambridge: Woodhead Publishing Limited. 2011; 2: 330-353
- Ayoka, A. O., Akomolafe, R. O., Akinsomisoye, O. S., et al. Medicinal and economic value of *Spondias mombin*. *Afr J Biomed Res.* 2008; 11: 129-136
- Ayoka, A. O., Akomolafe R. O., Iwalewa E. O., et al. Studies on the anxiolytic effects of *Spondias mombin* L. (Anacardiaceae) extracts. *Afr J Tradit Complement Altern Med.* 2005; 2: 153 - 165
- Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O., et al. Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiaceae) in mice and rats. *J Ethnopharmacol* 2006; 103: 166-175
- Akinmoladun, A. C., Obuotor, E. M., and Farombi, E. O. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. *J Med Food.* 2010; 13: 444- 451
- Aromolaran, O., and Badejo, O. K. Efficacy of fresh leaf extracts of *Spondias mombin* against some clinical bacterial isolates from typhoid patients. *Asian Pac J Trop Dis.* 2014; 4: 442 - 446
- Pragati, J., Poonam, K., and Rana, J.S. Green synthesis of zinc nanoparticles using flower extracts of *Nyctanthes arbor-tristis* and their antifungal activity. *J King Saud Univ. Sci.* 2018; 30: 168-175.
- Palaniselvam, K., Mashitah, M. Y., Gaanty, P. M., and Natanamurugaraj, G. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in Pharmacological applications - An updated report. *Saudi Pharm J.* 2016; 24: 473-484
- Logeswari, P., Silambarasam, S., and Abraham, J. Synthesis of Silver Nanoparticles using plant extracts and analysis of their antimicrobial property. *J Saudi Chem Soc.* 2015; 19: 311-317.
- Forough, M., and Farhad, K. Biological and Green Synthesis of Silver Nanoparticles. *Turkish J Eng Env Sci.* 2010; 34: 281-287
- Shahverdi, A. R., Fakhimi, A., Shahverdi H. R., and Minaian, S. Synthesis and effects of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *J Nanomedicine.* 2007; 3: 168-171
- Subhankari, I., and Nayak, P.L. Synthesis of Copper Nanoparticles using *Syzygium aromaticum*

- (Cloves) Aqueous Extract by Using Green Chemistry. World J Nano Sci Technol. 2013; 2: 14-17
18. Thatoi, P., Kerry, R. G., Gouda, S., Das, G., Pramanik, K., Thatoi, H., and Patra, J. K. Photo-mediated green synthesis of silver and zinc oxide nanoparticles using aqueous extracts of two mangrove plant species, *Heritiera fomes* and *Sonneratia apetala* and investigation of their biomedical applications. J Photochem Photobiol B. 2016; 163: 311-318
 19. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 26th informational supplement M100-S26, Wayne, PA: CLSI; 2016.
 20. Shah, R. K., Forishmeeta, B., and Nikahat, P. Synthesis and characterization of ZnO nanoparticles using leaf extract of *Camellia sinesis* and evaluation of their antimicrobial efficacy. Int J Curr Microbiol Appl Sci. 2015; 4: 444-450