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ASSESSMENT OF ANTIMICROBIAL ACTIVITIES OF ZINC NANOPARTICLES SYNTHESIZED FROM AQUEOUS LEAF EXTRACTS OF *OCIMUM GRATISSIMUM* AND *VERNONIA AMYGDALINA*

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ABSTRACT

Synthesis of nanoscale metals has been reported for environmental pollution effects and health problems which have made green synthesis via plant extracts to be preferred. As a viable alternative, use of plant extracts for effective synthesis of nano metallic particles has therefore been deployed due to safety and simple methodology. This study explored the potential of *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts as reducing agents for the synthesis of zinc nanoparticles. The extracts were utilized to reduce Zn^{2+} ions, and the resulting nanoparticles were characterized using UV-vis spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD). The XRD analysis revealed average particle size of 20.78 nm for nanoparticles synthesized with *Ocimum gratissimum* and 19.56 nm for *Vernonia amygdalina*. The antimicrobial properties of the synthesized nanoparticles were evaluated against *Bacillus thuringiensis (ATCC 789794.1), Staphyloccoccus aureus (ATCC 20923) and Escherichia coli (ATCC 25922)*. The results showed significant inhibition zones ranging from 8-19 mm for *O. gratissimum* and 6 – 18 mm for *V. amygdalina*-mediated nanoparticles. In comparison, doxycycline demonstrated 18-20 mm zones of inhibition, while deionized water showed no inhibitory effect. This study has demonstrated the effectiveness of the plants leaf extracts in synthesizing zinc nanoparticles with potent antimicrobial properties.

Keywords: Synthesis, Zinc-nanoparticles, *Ocimum-grantissimum*, *Vernonia- amygdalina*, characterization, antimicrobial properties.

INTRODUCTION

Nanotechnology has gained interest in research development. It deals with preparation and application of nanoparticles with varying sizes. Nanoparticles are useful in material science, industry, catalysis, diagnostic and microorganism control (Altammar, 2023, Paramo *et al*., 2020). Metallic nanoparticles have been reported as highly functioning nanomaterials that can act as antibacterial agents. Examples include, silver, gold and zinc nanoparticles (Husen, 2017). Zinc is essential for many biological activities in the human body (Parti *et al.,* 2023, Rajesh *et al*., 2015).

Silver nanoparticles green synthesis using Salvia spinosa plant extract (Pirtarighat *et al*., 2019) and zinc oxide nanoparticles using Cayratia pedata leaf extract (Ashwini *et al*., 2021) for examples have been reported. Syntheses of metallic nanoparticles via non green approaches are being contested owing to inherent toxicological effects (Adeyemi *et al.,* 2022, Amanda *et al*., 2010, Khan *et al.* 2021). Synthesis of nanoscale metals has been reported for environmental pollution effects and health problems which have made green synthesis via plant extracts to be preferred (Shuaixuan *et al*., 2022, Ying *et al*., 2022). As a viable alternative therefore, the use of plant extracts for effective synthesis of nano metallic particles has been deployed due to safety and simple method (Huang *et al*., 2007, Ullah and Lim, 2022). *Ocimum gratissimum* has been reported for therapeutic properties for which it has been considered antimicrobial, anti-cancer, antidiarrheal, antidiabetic, insecticidal, antiurolithiatic, antimutagenic (Sandeep, 2017). It has been reported for treatment of diarrhoea, conjunctivitis, cough and pneumonia (Adeniyi *et al*., 2012, Edo *et al.,* 2023). *Vernonia amygdalina*, a family of Asteraceae and genus *Vernonia* (Ebenezer and Olatunde, 2011) has been reported for microbial infections treatment (Abere and Yadessa, 2018), diabetes treatment (Ijeh and Ejike, 2011), malarial, febrifuge (Magadula and Erasto, 2009), healing wounds (Adetutu *et al*., 2011), treatment of infertility, canal issues, sexually transmitted diseases, protozoal infection (Farombi and Owoeye, 2011) and veneral diseases (Kambizi and Afolayan, 2001). It is of importance to note however, that plant extracts have been scarcely deployed for the synthesis of metal nanoparticles tailored towards antimicrobial applications despite the possible synergetic advantage of these extracts and zinc nanoparticles for improved antimicrobial activities.

Bioreduction ability of the phytochemicals components of the leaf extracts are to be utilized for the zinc nanoparticles synthesis after which, the antimicrobial propensity is tested on pathogens like *Bacillus thuringiensis, Staphyloccoccus aureus* and *Escherichia coli*. Hence, the effectiveness of the plants leaf extracts in synthesizing zinc nanoparticles with potent antimicrobial properties is hereby reported.

MATERIALS AND METHODS

Zinc nitrate was purchased from Fisher Co. while *O. gratissimum* and *V. amygdalina* leaves (Plate 1) were obtained at Federal University of Agriculture, Abeokuta, Nigeria.

(a) (b)

Plate 1. Plants: (a) *Ocimum gratissimum* (African basil), (b) *Vernonia amygdalina* (Bitter leaf)

Extract preparation

Fresh leaves were cleaned with water, cut into small pieces and soaked in 0.2 g/ml of distilled deionized water. The mixture was heated at 50 °C in water-bath for 20 minutes and was cooled and filtered (Henry *et al*., 2019). Extracts changed after heating from dark green to light brown for *Ocimum gratissimum* and dark green to brown for *Vernonia amygdalina*. The extracts were kept in a refrigerator for Zn nanoparticles synthesis, antimicrobial and other analysis.

Synthesis of zinc nanoparticles

Distilled deionized water of 20 mM zinc nitrate was prepared to produce 2 x 10^{-3}, 4 x 10^{-3}, 6 x 10^{-3}, 8 x 10^{-3} and 10 x 10^{-3} mole solutions. Leaf extract of 100 ml *Ocimum gratissimum* was added to 100 ml solution each at room temperature for bioreduction. *Vernonia amygdalina* extract similarly reacted, the solution was heated to 50 °C for 8 hr and cooled. Colour change was observed from light brown *O.gratissimum* extract to grayish black but brown *V.amygdalina* extract turned to gray-black for zinc nanoparticles solution centrifuged at 1000 rotation per minute for half an hr by using 720 N machine and lyophilized using DW-10N freeze dryer (Logeswari *et al*., 2015). Characterization and antimicrobial test followed.

Characterization of the zinc nanoparticles

The UV-Visible characterization involved determination of progress of reduction and the zinc nanoparticles synthesized due to reaction of Zn^{2+} ions with functional groups or chemical compounds of the plant extract. Reduction in the Zn^{2+} ions was examined and absorption spectra were obtained by using UV-Vis Shimadzu 200-800 nm spectrophotometer while distilled deionized water was used as blank. The zinc nanoparticles were also characterized with scanning electron microscope for agglomeration, morphology and particle distribution using SEM Techno A1 Camera in which little dried powder from prepared thin films zinc nanoparticles was analyzed on the Techno A1 Camera grid of copper coated with carbon. Zinc nanoparticles through thin film solution yielded patterns studied for the nature and particle size determination by using ADX-2700 XRD machine. Calculation of the zinc nanoparticle crystalline size was carried out on account of the XRD width obtained peaks and average size determined by using Debye-Scherrer equation (Narayanaswamy *et al*., 2015).

Antimicrobial test

Antimicrobial assay by agar well diffusion technique was carried out. Plates were seeded (100 µL) with each of the pathogenic isolates: *Bacillus thuringiensis* (789794.1), *Staphyloccoccus aureus* (ATCC 20923) and *Escherichia coli* (ATCC 25922). Each was treated with *Ocimum gratissimum, Vernonia amygdalina*, zinc nanoparticles (60 µL of 2 x 10^{-3}, 4 x 10^{-3}, 6 x 10^{-3}, 8 x 10^{-3} and 10 x 10^{-3} mole), doxycycline as positive and deionized water as negative control, replicated with mean values ± SD.

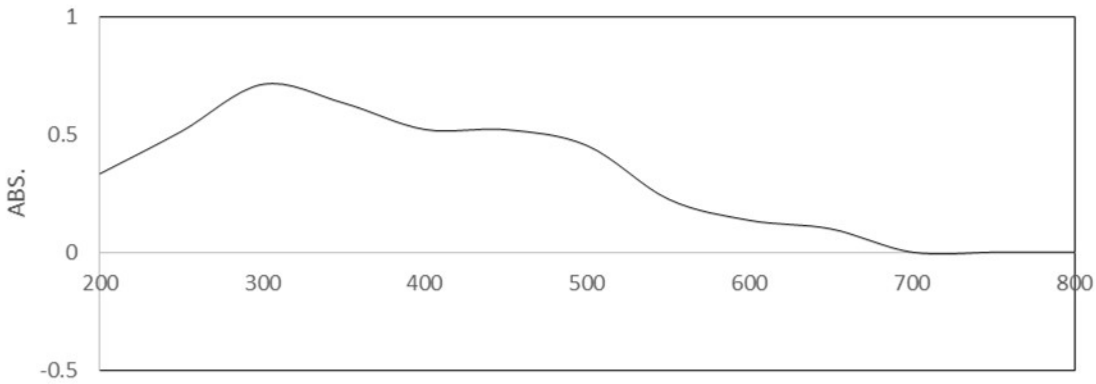
RESULTS

Colour of the *Ocimum gratissimum* leaf extract changed from dark green to light brown while that of the *Vernonia amygdalina* changed from dark green to brown after gentle heating. Extract of the *Ocimum gratissimum* leaves changed from light brown to grayish black when reacted with Z^{2+} ions in zinc nitrate solution. *Vernonia amygdalina* leaf extract turned gray-black from brown after its addition with zinc nitrate solution.

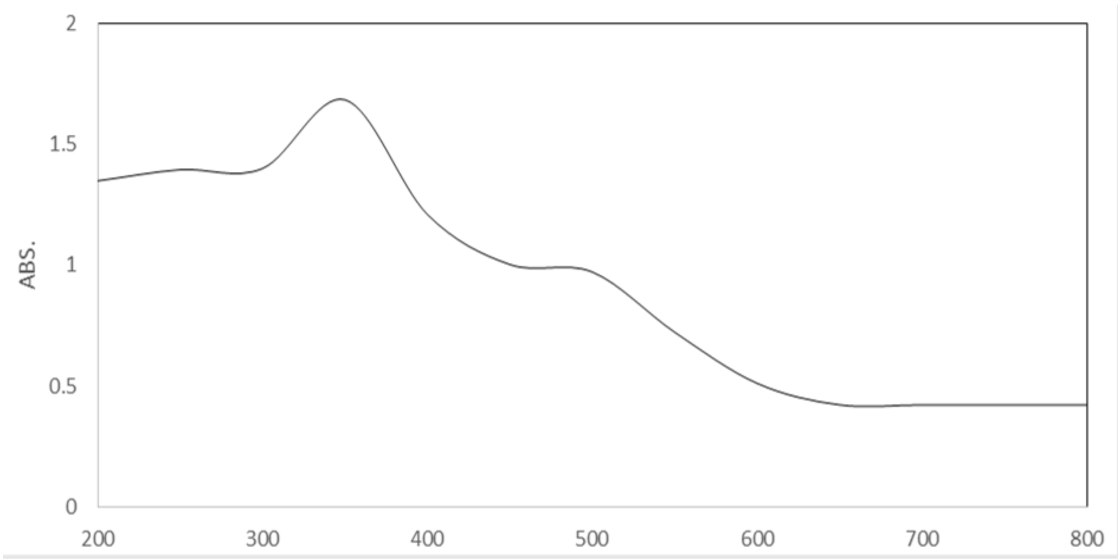
Uv-visible analysis

Absorption Spectra of the *Ocimum gratissi-*

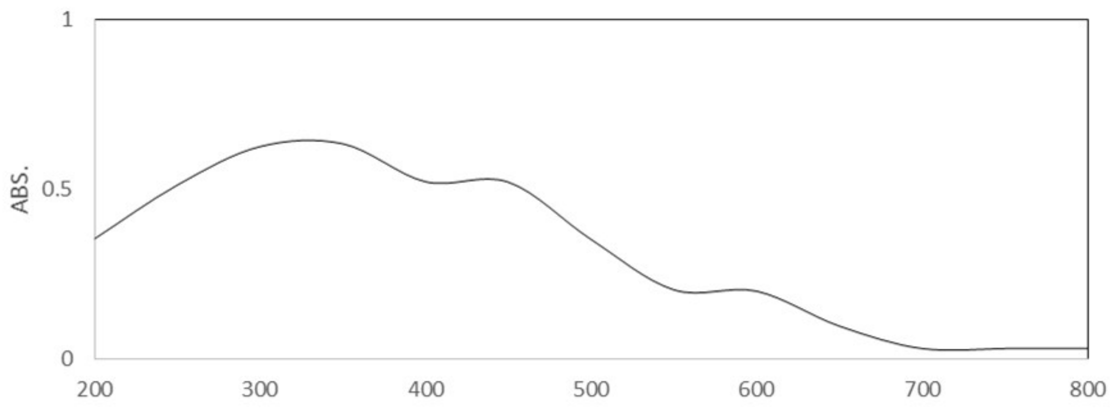
mum leaf extract and zinc nanoparticles synthesized with the leaf extract measured by using Uv-Visible Spectrometer showed absorption peaks at 310 and 360 nm (Figs. 1a and 1b) while the *Vernonia amygdalina* leaf extract and zinc nanoparticles showed absorption peaks at 300 and 340 nm (Figs. 1c, 1d) due to plasmon resonance as evidence for formation of Zinc nanoparticles (Ponarulselvam *et al.*, 2012).



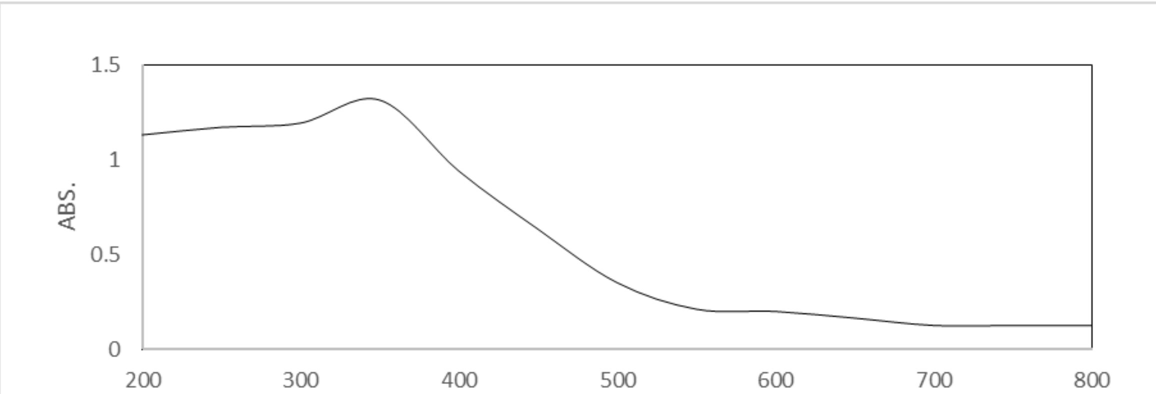
(a): ***Ocimum gratissimum*** **leaf extract**



(b) Zinc nanoparticles using leaves of *O. gratissimum*



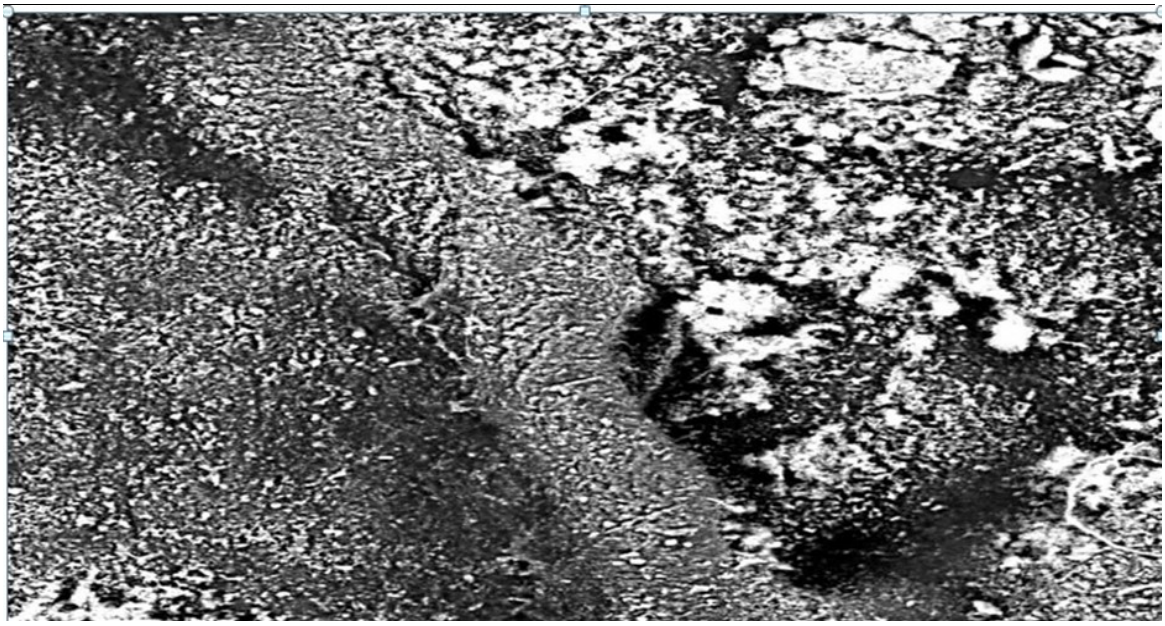
(c) *Vernonia amygdalina* leaf



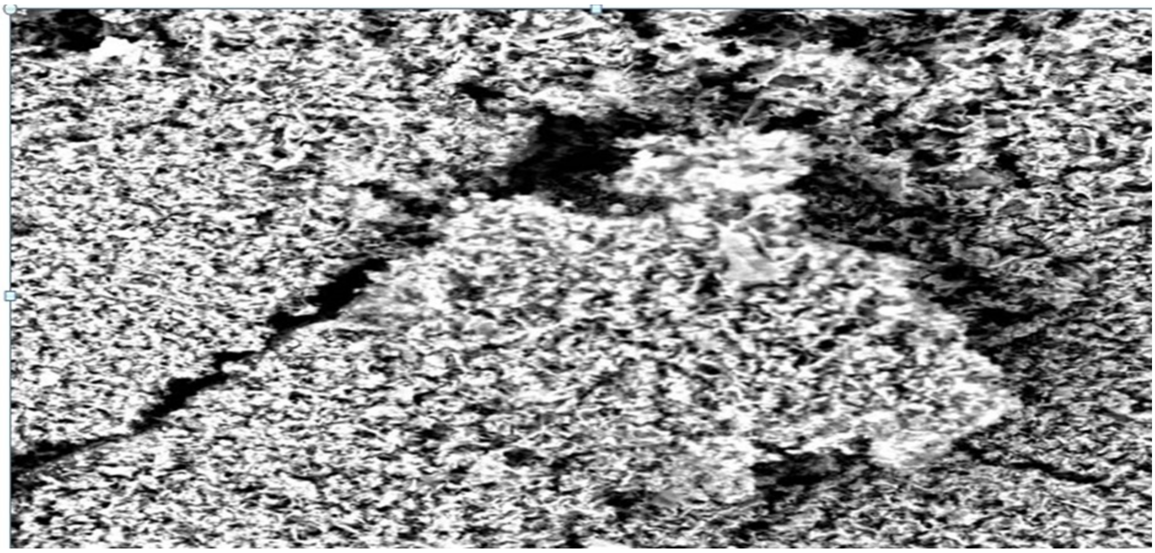
(d): Zinc nanoparticles using *Vernonia amygdalina* leaf

Figure. 1 Absorption Spectra (a) *Ocimum gratissimum* leaf
(b) Zinc nanoparticles using leaf of *O. gratissimum*
(c) *Vernonia amygdalina* leaf
(d) Zinc nanoparticles using *Vernonia amygdalina* leaf

Scanning electron microscope (SEM) SEM with agglomeration, morphology and particle distribution determination ability also revealed shape image of the zinc nanoparticles (Figs. 2a, 2b) with the *Ocimum gratissimum* and *Vernonia amygdalina* leaves extract by using Techno A1 Camera model.



(a)



(b)

Figure. 2: SEM image for:
(a) Zinc nanoparticles using *Ocimum gratissimum* leaf extract.
(b) Zinc nanoparticles using *Vernonia amygdalina* leaf.

X-ray diffraction analysis (XRD)
XRD analysis revealed size of the zinc nanoparticles formed from the *Ocimum gratissimum* and *Vernonia amygdalina* leaves extract. The indexed crystalline due to the Braggs reflection in the XRD pattern 34.4122°, 36.1355°, 42.4803°, 45.2318°, 56.1837°, 62.9235°, 68.2416° and 78.6635°

(Table 1a) were derived for zinc nanoparticles with *Ocimum gratissimum* while peaks 26.3735°, 28.2125°, 34.4122°, 36.1355°, 42.4803°, 45.2318°, 56.1837° and 62.9235° were indicated for the zinc nanoparticles synthesized with *Vernonia amygdalina* leaf extract (Table 1b). Average size for zinc nanoparticles with *Ocimum gratissimum* leaf extract evaluated by Debye Sherrer equation was 20.78 nm while average size for the zinc nanoparticles from *Vernonia amygdalina* leaf extract was 19.56 nm (Tables 1a, 1b).

Table 1: (a) XRD pattern: Zinc nanoparticles using *Ocimum gratissimum*

Position 2θ	FWHM	d-spacing (Å)	Height	Rel.int(%)	Area	Particle size
34.4122	0.313	4.5717	44	43.14	6	27.75
36.1355	0.348	4.2198	43	42.16	54	25.07
42.4803	0.498	3.3632	102	100.00	66	17.87
45.2318	0.489	2.8615	41	40.20	20	18.38
56.1837	0.512	2.0052	32	31.37	11	18.36
62.9235	0.546	1.6703	31	30.39	46	17.81
68.2416	0.498	1.5359	25	24.51	3	20.12
78.6635	0.512	1.4207	28	27.45	22	20.95

Calculated average zinc particle size: 20.78, FWHM = Full With at Half Maximum

(b) XRD pattern: Zinc nanoparticles using *Vernonia amygdalina*

Position 2θ	FWHM	d-spacing (Å)	Height	Rel.int (%)	Area	Particle size
26.3735	0.298	6.6152	32	31 .37	16.7	28.59
28.2125	0.431	5.4626	35	34.31	13.0	19.85
34.4122	0.489	4.5717	44	43.14	6.0	17.76
36.1355	0.487	4.2198	43	42.16	54.0	17.92
42.4803	0.498	3.3632	102	100.00	66.0	17.87
45.2318	0.489	2.8615	41	40.20	20.0	18.38
56.1837	0.512	2.0052	32	31.37	11.0	18.36
62.9235	0.546	1.6703	31	30.39	46.0	17.81

Calculated average zinc particle size 19.56

Antimicrobial Studies

The *Ocimum gratissimum* (Og) and *Vernonia amygdalina* (Va) leaves extracts and their zinc nanoparticles (Og-ZnNps) and (Va-ZnNps) showed inhibitory action against the selected microorganisms. *O. gratissimum* leaf extract showed 5 mm, 8 mm and 10 mm zone of inhibition against *Bacillus thuringiensis*-ATCC 789794.1 (B.t)*, Staphyloccoccus aureus*-ATCC 20923 (Sa) and *Escherichia coli*-ATCC 25922 (Ec) respectively while *V. amygdalina* (Va) leaf extract demonstrated 6 mm, 9 mm and 11 mm zone of inhibition against the *Bacillus thuringiensis*-ATCC 789794.1 (Bt), *Staphyloccoccus aureus*-ATCC 20923 (Sa) and *Escherichia coli*-ATCC 25922 (Ec) in the same order. Higher zone of inhibitory action ranged from 8 mm to 19 mm were observed for the Zinc - *Ocimum gratissimum* nanoparticles (Og-ZnNps) against *Bacillus thuringiensis*-ATCC 789794.1, *Staphyloccoccus aureus*-ATCC 20923 and *Escherichia coli*-ATCC 25922 compared with 6 mm to 18 mm range of inhibition action demonstrated by the zinc nanoparticles synthesized with *Vernonia amygdalina* (Va- ZnNps) leaves extract against the *Bacillus thuringiensis*-ATCC 789794.1, *Staphyloccoccus aureus*-ATCC 20923 and *Escherichia coli*-ATCC 25922. The inhibitory action of the zinc nanoparticles from both the *Ocimum gratissimum* and *Vernonia amygdalina* leaves extract was strongly comparable with zone of inhibition exhibited by the positive control doxycycline ranged from 18 mm to 20 mm against the *Bacillus thuringiensis*-ATCC 789794.1, *Staphyloccoccus aureus*-ATCC 20923 and *Escherichia coli*-ATCC 25922. The distilled deionized water (Ddw) did not demonstrate any action of inhibition which confirmed that the inhibitory action was due to the antimicrobial activity of the plant extract and the zinc nanoparticles (Table 2).

Table 2: Antimicrobial inhibition activity of the leaves and zinc nanoparticles

Sample	Microorganism			Samples	Microorganism		
(Concentration)	Bt	Sa	Ec				
(Concentration)	Bt	Sa	Ec	60 µL			
Zone of inhibition (mm)			60 µL				
Zone of inhibition (mm)							
Og	5	8	10	*Va*	4	6	8
Og-ZnNPs	Zone of inhibition (mm)			*Va*-ZnNP	Zone of inhibition (mm)		
.2 x10^{-2} M	8	11	12	.2 x10^{-2} M	6	9	11
.4 x10^{-2} M	10	13	14	.4 x10^{-2} M	8	10	13
.6 x10^{-2} M	11	14	15	.6 x10^{-2} M	11	13	14
.8 x10^{-2} M	14	16	17	.8 x10^{-2} M	13	15	16
1. x10^{-2} M	16	17	19	1. x10^{-2} M	15	16	18
Doxycycline	18	20	20	Doxycycline	19	20	20
D.d.w	0	0	0	D.d.w	0	0	0

Bt = *Bacillus thuringiensis*; Sa = *Staphyloccoccus aureus*; Ec = *Escherichia coli*; Og = *Ocimum gratissimum*; Va = *Vernonia amygdalina*; ZnNPs = Zinc nanoparticles
The analysis was carried out three times, average values ± SD was recorded.

DISCUSSION

The colour change that resulted from reduction reaction due to different metabolites from the leaves extracts revealed complex formation of the Zinc nanoparticles (Pirtarighat et al., 2019). The observed colour change also indicated occurrence of surface plasmon vibrations in excited state while the broad peaks demonstrated particles distribution as proof for zinc complex formation (Pirtarighat et al., 2019). Colourless to yellowish brown change in silver nanoparticles has been reported (Narayanaswamy *et al.*, 2015b). Pale yellow turned to pale brown which demonstrated zinc oxide nanoparticles production from Camellia sinesis has been reported (Rajesh *et al.*, 2015). Change in colour from bright yellow to dark brown as a proof of silver nanoparticles synthesis with plant extract has also been reported (Logeswari *et al.*, 2015).

The broad spectral observed from the UV–Vis analysis could be attributed to the broad plasmon band characteristic of plant extract metabolites within that spectrophotometric range while the single surface plasmon resonance proved spherical shape of the zinc nanoparticles. Increase in peaks resulted from changes in particle shapes which described spherical trend (Banerjee *et al.*, 2014) that gave clear evidence that the zinc nanoparticles were formed. The UV-Vis spectral demonstrated the need for plant leaf chemical compound sufficient availability for the zinc nanoparticles maximum production. Surface plasmon vibrations excitation has been reported (Ponarulselvam *et al.*, 2012). Absorption spectrum of Zinc oxide has shown absorbance peak at 355 nm and 320 nm (Ashwini *et al.*, 2021).
The particle and aggregate details of the nanoparticles were revealed by the SEM image. Uniform distribution of the nanoparticles was observed while Morphological examination indicated separation among the particles with proof of spherical shapes. Result of the SEM analysis agreed with spherical morphology between 10 and 50 nm (Narayanaswamy *et al.*, 2015) and nanoparticles spherical shape from *Boswellia ovalifoliolata* in the range of 30 to 40 nm (Savithramma et al., 2011).

The XRD analysis confirmed the zinc nanoparticles formation through demonstration of the planes Braggs reflection of indexed crystalline zinc particles structure. Appearance of the peaks suggested spherical nature of the nanoparticles even in the presence of unassigned peaks attributable to bioorganic contribution. The XRD result was in agreement with 16 nm synthesized zinc oxide nanoparticle reported (Senthilkumar and Sivakumar, 2014.) and another reported using *Lippia adoensis* leaf extract (Meron et al., 2020).

Antibacterial assay of synthesized nanoparticles used against urinary infection pathogen has been reported (Santhoshkumar *et al.*, 2017). Bactericidal effect of nanoparticles synthesized by using hibiscus subdariffa leaf extract has been reported (Bala et al., 2015). Nanoparticles with antibacterial activity synthesized by using *Lippia adoensis* leaf extract has been reported (Meron et al., 2020). Non-detection of antimicrobial inhibitory effect of the distilled deionized water used as negative control (Pirtarighat et al., 2019) and also used as blank suggested that the inhibitory activities recorded were demonstrated by the extracts and the zinc nanoparticles. The inhibition activity of the zinc nanoparticle which was examined to be higher than those of the *Ocimum gratissimum* and *Vernonia amygdalina* extracts could be attributed to metal ion effect on the microorganisms cell wall (Godoy-Gallardo *et al* 2021). Differnce in the nanoparticles inhibition effect against the *Bacillus thuringiensis* (ATCC 789794.1), *Staphyloccoccus aureus* (ATCC 20923) and *Escherichia coli* (ATCC 25922) might resulted from differnce in microorganism resistance (Uddin *et al*., 2021).

CONCLUSION

Metals including zinc could be substituted for more costly metals like silver and gold in nanoparticles synthesis with plant extracts due to their relative relevant particles properties in the field of nanotechnology. Simple eco-friendly method, cost effectiveness, reliable materials and numerous areas of application have made nanoparticles synthesis acceptable. Nanoparticles are not only important in nanotechnology but also in medicine. Bioreduction ability utilized chemical components of these leaves extract in the zinc nanoparticles synthesis. This synthesis is not only simple but efficient, economical and revealed that the zinc nanoparticles demonstrated antimicrobial property strongly comparable with standard antibiotic.

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