IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 13, Issue 8 Ser. I (August. 2020), PP 14-21
www.iosrjournals.org

ZnO Nanoparticles (ZnO-NPs): Synthesis Using *Tithonia diversifolia*, Characterization and *in-vitro* Antimicrobial Bioassays

Bonface Juma Wafula[*1], Eric Masika[1] and Charles Onindo[1]
[1]*Department of Chemistry, Kenyatta University, Kenya*
** Corresponding author*

Abstract
ZnO is among leading metal oxide nanoparticles (MO-NPs). The conventional physical and chemical synthesis protocols require use of sophisticated equipment and many chemicals which may be hazardous. In this research, ZnO-NPs were prepared via a straightforward environmentally benign technique; employing Tithonia diversifolia as a reductant. Zinc nitrate solution was mixed with TDLE in the ratio of 3:1 respectively and incubated in an ultra-sonication bath. The precipitates were centrifuged at 5000 rpm for 15 minutes followed by washing with distilled water. A peak at 374 nm, as measured by UV-Vis, confirmed formation of nano-ZnO.Presence of ZnO was shown by a new peak at 668.35 cm^{-1} in the ZnO-TDLE FTIR spectrum. The crystalline size of the sample was estimated as 20.91 nm by Debye-Scherer formula. Identification and quantification of elements was performed by X-Ray Fluorescence (XRF) analysis, which showed 91.823% Zn in the sample. The synthesized nano-ZnO showed promising antibacterial activity. The bioactive nature of ZnO-NPs, as demonstrated in this research, puts the nano-ZnO at pole position for the formulation of novel antimicrobial agents.

Keywords: *antimicrobial activity; Tithonia diversifolia; XRD; X-Ray Fluorescence; ZnO-NPs*

Date of Submission: 01-08-2020 Date of Acceptance: 16-08-2020

I. Introduction

ZnO is currently among the most fascinating MO-NPs [1, 2]because of its unique chemical and physical properties [3]. No wonder, it is applied widely[4]in fields such as nanomedicine [5],chemistry, agriculture [6],environment [7]and technology. Consequently, there is need of exploring new synthesis protocols; besides establishing a cutting-edge understanding of its properties.

Physical methods of synthesizing MO-NPsare well established but their utilization to fullness has remained elusive due to requirement of sophisticated equipment and scaling-up challenges. Although chemical methods are cheaper, fast and easy to scale up; but they involve use of many reagents [8, 9]. Biological synthesis which is considered eco- friendly [1-2, 4-7], fast, clean[10] and less-costly [11]is currently being explored. Plant extracts, living matter residue, wastes, bacteria and fungi have been found to aid in formation of ZnO-NPs as reducing and stabilizing agents. Synthesis aided by plant extracts is preferred [9] because(i) plant extracts can be found in plenty, (ii) it is easier scaling up production and (iii) it is a one-step synthesis.

Plants contain phytochemicals [4, 7] that act as reductants as well as stabilizing agents [11]. Using environmentally benign materials [12-13] like leaf extract for synthesis of safer and biocompatible MO-NPs [1] is gaining currency. ZnO NPs of various shape, size and properties have been synthesized. In one research, zinc acetate and *Azadirachta indica* yielded nano-ZnO (25 nm) [14].Mushtaq and coworkers synthesized ZnO-NPs (14.18 nm) using *Rubia cordifolia*[15]. The synthesized NPs wereactive against *S. aureus* and *E. coli*. These studies have proven the efficacy of plant extracts as reductants [16]in synthesis of MO-NPs.

The demand for cheaper, safer and biocompatible ZnO-NPs overwhelmingly outstrips the current supply. Use of plants as reductants for synthesizing MO-NPs has emerged as a favorite area of research. Various plants such as *Moringa oleifera*[1],*Calotropis gigantean* [6],*Pentatropis capensis*[10],*Pongamia pinnata*[13], and *Camellia sinesis*[17]have been used to prepare ZnO-NPs; it will be interesting to see how *T. diversifolia* would assist in the preparation of ZnO-NPs.

This research thus deals with rapid preparation of ZnO-NPsusing TDLE and evaluation of their antimicrobial properties. Surge in drug resistance and emergence of infectious diseases, calls for urgent formulation of novel MO-NPs as bioactive agents [16-18].

II. Materials and Methods

2.1 Materials

T. diversifolia was randomly sourced fromMt. Elgon, Kenya. Zinc nitrate hexahydrate and NaOH (analytical grade) were obtained from Merck Co. Dimethyl sulphoxide (DMSO) was purchased locally.

Photo 1: *Tithonia diversifolia* (photo taken by Bonface Juma)

2.2 Methods

2.2.1 Preparation of Leaf Extract of Tithonia diversifolia

The extract was prepared following the protocol employed in past research [19]. The leaves were washed with distilled water and dried overnight at 70˚C. They were then ground into powder and 8 g of the crushed sample dissolved in 100 ml of distilled water in 250 ml round-bottomed flask. The mixture was heated at 70˚C for half an hour. It was cooled and then filtered to obtain TDLE which was preserved at about 2˚C.

2.2.2 Preparation of nano-ZnO Particles

Exactly 150 ml of zinc nitrate (0.06 M) was briefly agitated and 50 ml of TDLE (mixing ratio of 3:1) added followed by drops of 2M NaOH to raise pH to 10. The mixture was incubated in an ultra-sonication bath at 80˚C. After 2h, the precipitate was washed three times in a centrifuge (5000 rpm for 15 minutes). It was dried at 100˚C for 24 h and stored at room temperature.

2.2.3 Characterization of the Samples

Properties of nano-ZnO were studied by XRD [15, 20], UV-Vis [9, 21], FTIR[4]and XRF [21].

2.2.4 Antibacterial Bioassays

The antibacterial bioassay was performed by agar disc diffusion method [1]. Discs were soaked in 150 µl[15]ZnO suspension (in 0.5% dimethyl sulphoxide) and placed onto agar plates containing the microbes. The plates were stored at 37 °C for 24 h [1]. Positive controls (Gentamicin, Ampicillin, Penicillin, Streptomycin and Ciprofloxacin) and negative controls (TDLE and DMSO) were used. Zone diameters were measured using a ruler. The bioassays were carried out in triplicate.

III. Results

3.1 Colour Changes

When colourless zinc nitrate was added to dark brown TDLE, the mixture turned to green yellow within five minutes signifying possible conversion of Zn (II) ions to Zn [10]. After 2 h the mixture eventually turned to an off-white colour, an indication of nano-ZnO formed.

Figure 1: Synthesis of ZnO NPs using TDLE; I- TDLE, II- incubation in a sonicator, III- after 2 h and IV- synthesized ZnO NPs.

3.2 UV-Vis Analysis

Formation of MO-NPs can be confirmed by UV-Vis measurements [21]. In this study, UV-Vis Spectrophotometer model SPECORD 200 PLUS (analytikjena), Germany model was used.Under optimized conditions, maximum absorption (λ_{max}) was observed at 374.0 nm. This is in line with previous studiesinvolving synthesis of ZnO-NPs, where λ_{max} =372.0 nm [9] was observed. In another research, similar results were obtained where λ_{max} was found to be at 370.0 nm [22]. The energy band gap (3.316 eV) was determined using equation 1 [9]. This value agrees with the theoretical band gap of ZnO which is reported as 3.37 [23].

Energy band gap = $\frac{1240}{\lambda_{max}}$ …… Equation 1

Time was used to optimize the reaction; whereby increase in time from 10-120 minutes led to sharpening of peaks [5]. A sharp peak with high absorbance was realized at 120 minutes.Alkaline solutions are known to aid faster formation of small-sized NPs. In this work, increase of pH from 8-10 was accompanied by sharpening of peaks and increase in absorbance. Maximum absorbance was realized at pH=10. Increasing concentration of $Zn(NO_3)_2$ from 0.06 to 0.1 M witnessed increase in peak broadening. Thus, increasing Zn^{2+} beyond threshold [5] hinders formation of ZnO-NPs. Mixing $Zn(NO_3)_2$ and TDLE in the ratio of 3:1 respectively resulted into a sharp peak with increased absorbance. Mass of TDLE per 100 ml of water witnessed a sharp peak and higher absorbance at 8g.

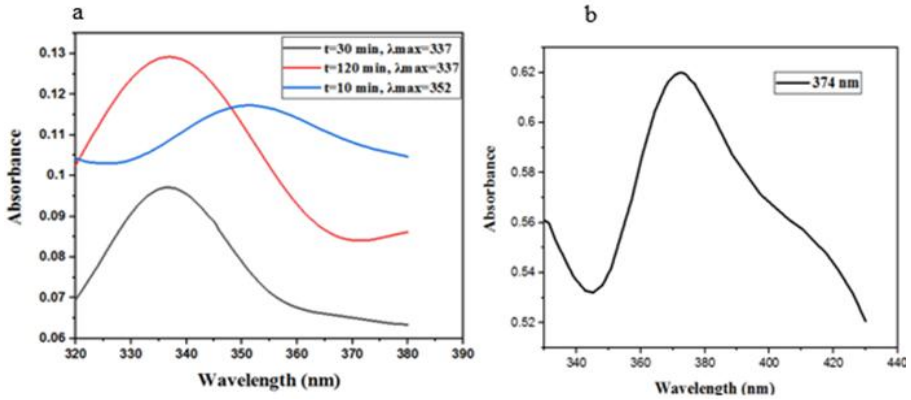


Figure 2:Spectrum (UV-Vis) of ZnO-TDLE-NPs; a. at different reaction times and b. at optimized conditions

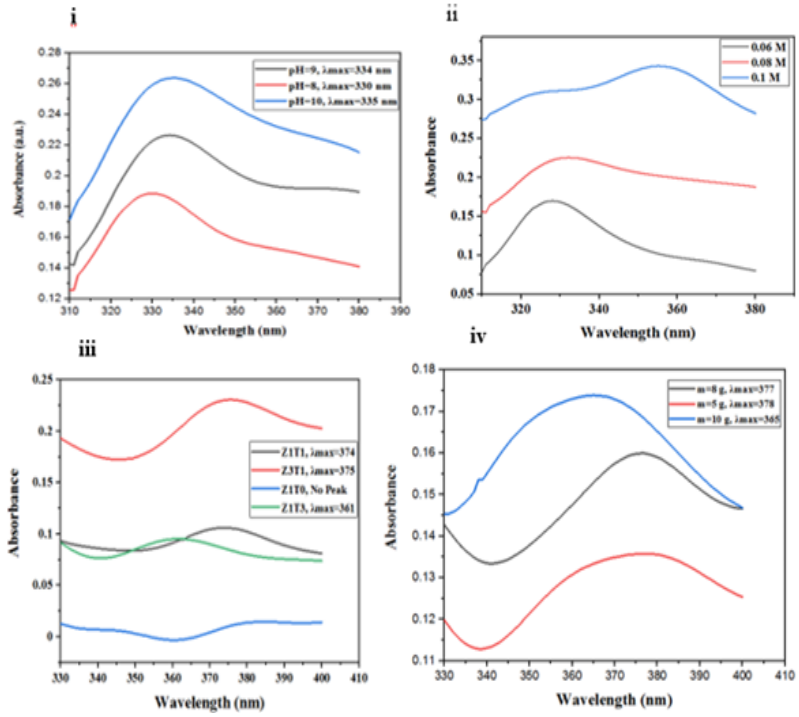


Figure 3: Optimization of parameters; i. pH, ii. concentration of $Zn(NO_3)_2$, iii. mixing ratios and iv. mass of *T. diversifolia*per 100 ml water

3.3FTIR Characterization

The FTIR spectrum was scanned from 4000 cm^{-1} to 500 cm^{-1} using FTIR spectrophotometer model IRTracer (SHIMADZU). The shifting, disappearance, broadening and emergence of new peaks revealed participation of phytochemicals in the synthesis of ZnO-NPs. A strong IR peak at 3282.9 cm^{-1} assigned to –OH stretch[19]shifted to 3428.53 cm^{-1}. There was emergence of a new small peak at 2359.95 cm^{-1}. A peak at 1634.7 cm^{-1}, assigned to C= C, shifted to a lower wavenumber of 1560.44 cm^{-1}. New peaks emerged at 668.35, 918.13 and 1029.04 cm^{-1} which could be assigned to ZnO stretch.

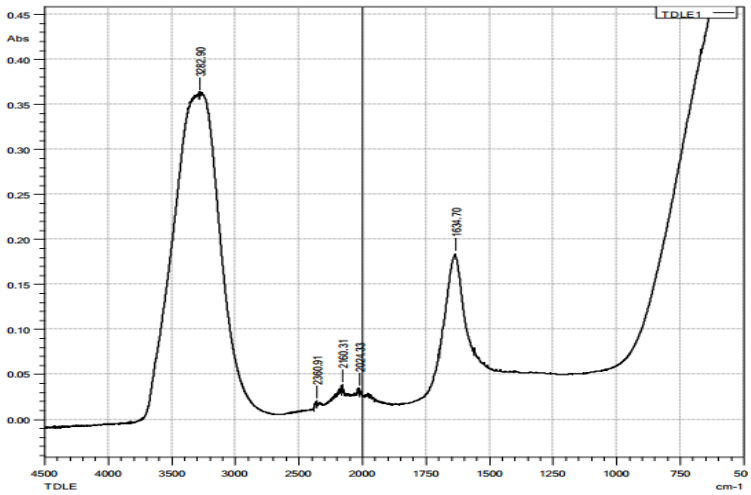


Figure 4: FTIR spectrum of TDLE

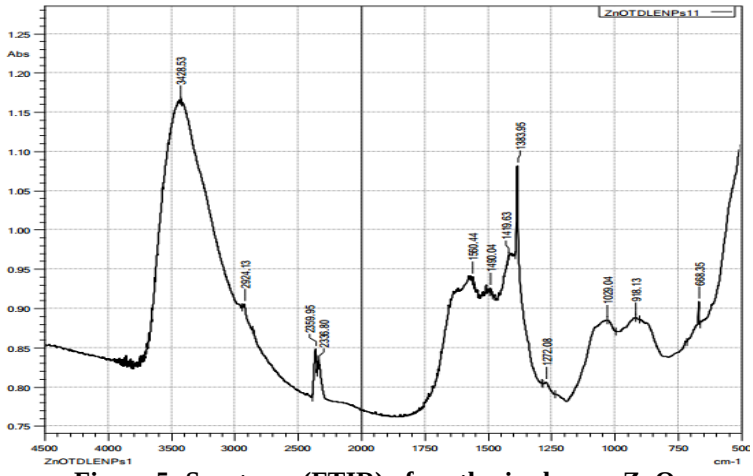


Figure 5: Spectrum (FTIR) of synthesized nano-ZnO

3.4XRD

The synthesized nano-ZnO was hexagonal wurtzite [21]and highly crystalline with crystalline size estimated as 20.91 nm (from most intense peak) using Debye-Scherer formula [5, 20].

Table 1: Summarized properties of ZnO-TDLE-NPs

Lattice parameters	a=3.22461Å, b=3.22461Å, c=5.19180Å
Angles	α=90˚, β=90˚, γ=120˚
Space group number and name	186, P63mc

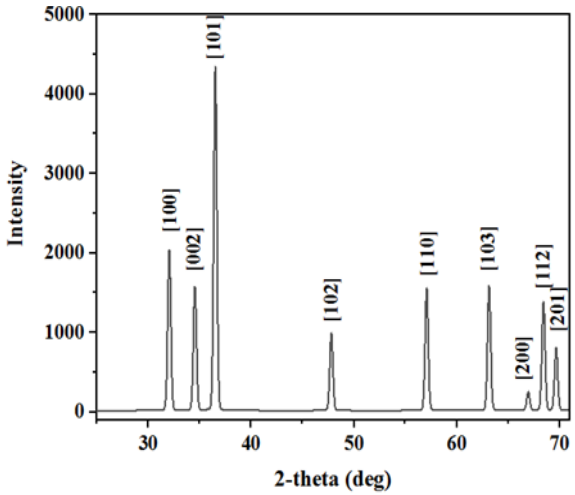


Figure 6: XRD scan of the synthesized ZnO-NPs

3.5X-Ray Fluorescence Analysis

XRF was used for identification and quantification of elements in the sample, whereby 91.823% was assigned to Zn. The high content of Zn in the sample is a clear indication of complete reduction of zinc nitrate to ZnO-NPs and also absence of impurities. This research, in fact, reports a much higher content of Zn compared to 14.96% reported earlier by Khaing and co-workers [21].

Table 2: Elemental analysis of ZnO-TDLE NPs by XRF

BRUKER

00884-GeoChem.pdz	AssayTime: 01/04/2019 13:16:37	ElapsedTime: 18	
Alloy 1:	Match No:		
Field Info			
Operator	USER	lab no	
refference	ZnTDLE nPS 3	Name	

Element Name	Min	%	Max	+/- [*3]
MgO	0	0.000	0	2.065
Al2O3	0	0.504	0	0.272
SiO2	0	0.486	0	0.163
P2O5	0	0.314	0	0.178
S	0	0.000	0	0.074
Cl	0	0.000	0	0.122
K2O	0	0.054	0	0.017
CaO	0	0.152	0	0.023
Ti	0	0.045	0	0.012
V	0	0.031	0	0.010
Cr	0	0.072	0	0.009
Mn	0	0.000	0	0.000
Fe	0	0.000	0	0.000
Co	0	0.000	0	0.025
Ni	0	0.184	0	0.058
Cu	0	0.000	0	0.019
Zn	0	91.823	0	0.754
As	0	0.000	0	0.007
Se	0	0.000	0	0.005
Rb	0	0.000	0	0.006
Sr	0	0.000	0	0.007
Y	0	0.000	0	0.005
Zr	0	0.000	0	0.006
Nb	0	0.000	0	0.011
Mo	0	0.305	0	0.027
Pd	0	0.000	0	0.003
Ag	0	0.000	0	0.014
Cd	0	0.000	0	0.026
Sn	0	0.000	0	0.005
Sb	0	0.000	0	0.046
Ba	0	0.000	0	0.145
La	0	0.000	0	0.404
Ce	0	0.514	0	0.074
Hf	0	0.000	0	0.031
Ta	0	0.000	0	0.166
W	0	0.000	0	0.296
Pt	0	0.000	0	0.078
Au	0	5.515	0	0.447
Hg	0	0.000	0	0.025
Tl	0	0.000	0	0.015
Pb	0	0.000	0	0.109
Bi	0	0.000	0	0.018
Th	0	0.000	0	0.028
U	0	0.000	0	0.160

3.6Antimicrobial Potential of the Synthesized ZnO NPs

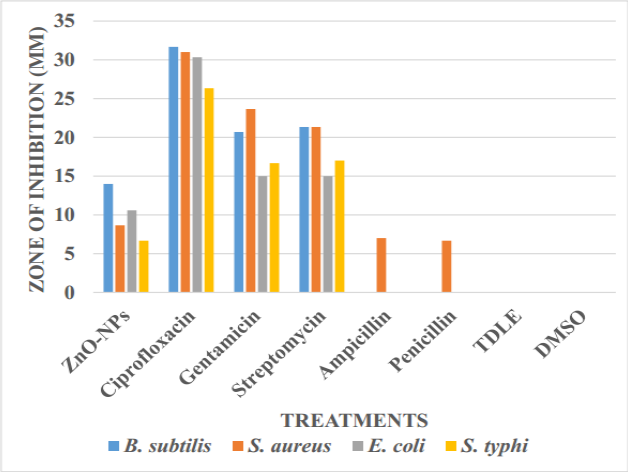
One-way ANOVA was used to analyze data using JASP software version 0.12.2. The highest activity (mm) was reported against *B. subtilis* (14.0±1), a Gram positive while the lowest was reported against *S. typhi*(6.67±0.58), a Gram negative.The activity of ZnO-NPs against *E. coli*was reported as 10.6±0.58 mm; this agrees well with results earlier reported [24]. It is interesting to note that the current research observed higher antibacterial activity than previous studies done by Fatimah (2018) [20] and Meruvu et al. (2011) [25]. Elsewhere, researchers analyzed antimicrobial potential of ZnO NPs against *E. coli* using agar well diffusion [15]. They obtained zone inhibitions of 12, 14 and 15 mm at 70, 100 and 150 µl of ZnO NPs respectively.

Table 3: Antibacterial bioassays

Treatment	**Zonediameter±SD (mm)**			
	B. subtilis	***S. aureus***	***E. coli***	***S. typhi***
ZnO NPs	14.0 ± 1^{f}	8.67 ± 0.58^{h}	10.6 ± 0.58^{g}	6.67 ± 0.58^{i}
Ciprofloxacin	31.67 ± 0.58^{a}	31.0 ± 1^{a}	30.33 ± 0.58^{a}	26.33 ± 1.53^{b}
Gentamicin	20.7 ± 0.58^{d}	23.67 ± 0.58^{c}	15.0 ± 1^{e}	16.67 ± 0.58^{e}
Streptomycin	21.33 ± 1.53^{cd}	21.33 ± 2.31^{c}	15.0 ± 1^{e}	17.0 ± 1^{e}
Ampicillin	-	7.0 ± 1.73^{h}	-	-
Penicillin	-	6.67 ± 1.16^{h}	-	-
TDLE	-	-	-	-
DMSO	-	-	-	-

-: No antibacterial activity reported.

Note: means followed by the same superscript (a-i) in the same column or row are not significantly different as determined by one-way ANOVA at 95% confidence levels.



Graph 1: Antimicrobial activity of ZnO-TDLE-NPs and its Control Treatments

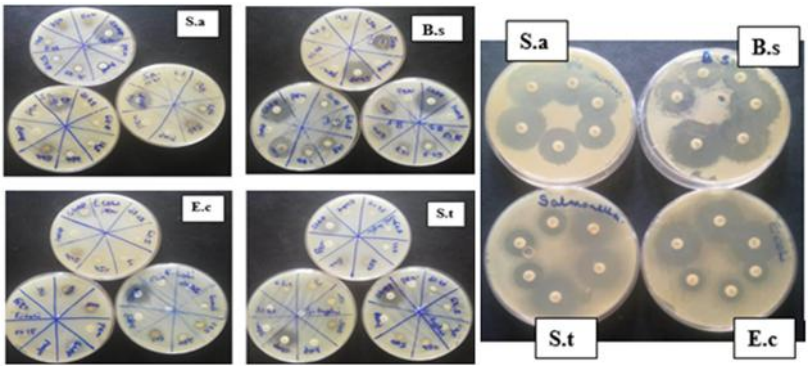


Plate 1: Zone of inhibition of ZnO-NPs, Streptomycin, Penicillin, Ampicillin, Ciprofloxacin, Gentamicin, TDLE and DMSO against- S.a (*S. aureus*), B.s (*B. subtilis*), E.c (*E. coli*) and S.t (*S. typhi*)

IV. Discussion

Size, shape, chemical composition and even the type of plant extract used determine the efficacy of MO-NPs inhibiting growth of microbes. *T. diversifolia* has been demonstrated to aid in the formation of ZnO-NPs (20.91 nm) with antimicrobial potential.ZnO-NPs are much smaller as compared to bacteria

[26];consequently, it is much easier for them to stick to the cell membrane of bacteria, damaging it and eventually death of the cell. Formation of ROS (H_2O_2, OH^-, O_2&O_2^-) is thought to be the main antibacterial activity mechanism [27]. ROS species react with H^+ to yield H_2O_2[26] which can easily cross into the cell via the cell membrane. The entry of H_2O_2 into the bacteria cell compromises the genetic materials; eventually the cell dies.

Table 4: Comparison studies

Method	Precursors	Crystalline size (nm)	Antimicrobial activity (mm)	References
Biological	Zinc nitrate, *Tithonia diversifolia*	20.91	*S. aureus*-8.67, *B. subtilis*-14, *E. coli*-10.6, *S. typhie*-6.67	Current study
Biological	Zinc nitrate, *Rubia cordifolia*	14.18	*S. aureus*-15, *E. coli*-15	[15]
Biological	Zinc acetate dihydrate, rice bran	17.76	*S. aureus*-7.3, *E. coli*-7.6	[20]
Chemical (Precipitation)	Zinc acetate, ammonium carbonate	30.00	*B. subtilis*-8, *E. coli*-7	[25]

V. Conclusion

A 'green' method for preparation of ZnO-NPs through bio-reduction of Zn^{2+} using TDLE was demonstrated. FTIR confirmed participation of phytochemicals as reductants. The obtained nano-ZnO was highly crystalline with crystalline size of 20.91 nm. The synthesized ZnO-NPs particles showed promising antimicrobial potential.

Acknowledgements

We acknowledge Ms. Jane Mburu and Ms. Catherine Wanja, both of the department of Chemistry at Kenyatta University, Kenya for their guidance during laboratory work.

References

[1]. Pal, S., Mondal, S., Maity, J., & Mukherjee, R. (2018). Synthesis and characterization of ZnO nanoparticles using *Moringa oleifera* leaf extract: investigation of photocatalytic and antibacterial activity. *InternationalJournal of Nanoscience and Nanotechnology*, 14(2), 111–119.

[2]. Kalpana, V. N., Rajeswari, V. D., & Fanizzi, F. P. (2018). A Review on Green Synthesis, Biomedical Applications, and Toxicity Studies of ZnO NPs. Bioinorganic Chemistry and Applications, 2018, 12. https://doi.org/10.1155/2018/3569758

[3]. Kolodziejczak-Radzimska, A., & Jesionowski, T. (2014). Zinc oxide-from synthesis to application: A review. Materials, 7(4), 2833–2881. https://doi.org/10.3390/ma7042833

[4]. Fakhari, S., Jamzad, M., & Kabiri Fard, H. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. *Green Chemistry Letters and Reviews*, *12*(1), 19–24. https://doi.org/10.1080/17518253.2018.1547925

[5]. Jamdagni, P., Khatri, P., & Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. *Journal of King Saud University - Science*, *30*(2), 168–175. https://doi.org/10.1016/j.jksus.2016.10.002

[6]. Chaudhuri, S. K., & Malodia, L. (2017). Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: Characterization and its evaluation on tree seedling growth in nursery stage. *Applied Nanoscience (Switzerland)*, *7*(8), 501–512. https://doi.org/10.1007/s13204-017-0586-7

[7]. Bharati, R., & Suresh, S. (2017). Biosynthesis of ZnO/SiO_2 nanocatalyst with palash leaves' powder for treatment of petroleum refinery effluent. *Resource-Efficient Technologies*, *3*(4), 528–541. https://doi.org/10.1016/j.reffit.2017.08.004

[8]. Gudikandula, K., & Charya Maringanti, S. (2016). Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. *Journal of Experimental Nanoscience*, *11*(9), 714–721. https://doi.org/10.1080/17458080.2016.1139196

[9]. Happy, A., Soumya, M., Venkat Kumar, S., Rajeshkumar, S., Sheba Rani, N. D., Lakshmi, T., & Deepak Nallaswamy, V. (2019). Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*. *Biochemistry and Biophysics Reports*, *17*(December 2018), 208–211. https://doi.org/10.1016/j.bbrep.2019.01.002

[10]. Surekha, K., Abirami, R., Prasidhee, V., & Jacob, S. J. P. (2017). Green synthesis of zinc oxide nanoparticle using *Pentatropis capensis* and its anti-proliferative activity. *Indian Journal of Natural Products and Resources*, *8*(4), 316–321.

[11]. Jain, N., Bhargava, A., & Panwar, J. (2014). Enhanced photocatalytic degradation of methylene blue using biologically synthesized "protein-capped" ZnO nanoparticles. *Chemical Engineering Journal*, *243*, 549–555. https://doi.org/10.1016/j.cej.2013.11.085

[12]. Patil, B. N., & Taranath, T. C. (2016). Limonia acidissima L. leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against *Mycobacterium tuberculosis*. *International Journal of Mycobacteriology*, *5*(2), 197–204. https://doi.org/10.1016/j.ijmyco.2016.03.004

[13]. Sundrarajan, M., Ambika, S., & Bharathi, K. (2015). Plant-extract mediated synthesis of ZnO nanoparticles using *Pongamia pinnata* and their activity against pathogenic bacteria. *Advanced Powder Technology*, *26*(5), 1294–1299. https://doi.org/10.1016/j.apt.2015.07.001

[14]. Oudhia, A., Kulkarni, P., & Sharma, S. (2015). Green Synthesisof ZnO nanotubes for Bioapplications. *Proceedings of BITCON-2015-International Journal of Advanced Engineering Research and Studies*, 280–281.

[15]. Mushtaq, A., Patel, R., Singh, N., Negi, D. S., & Rawat, S. (2017). Green synthesis of zinc oxide nanoparticles using *Rubia cordifolia* root extract against different bacterial pathogens. *Indo American Journal of Pharmaceutical Research*, *7*(9), 759–765

[16]. Uikey, P., & Vishwakarma, K. (2016). Review of zinc oxide (ZnO) nanoparticles applications and properties. In *International Journal of Emerging Technology in Computer Science & Electronics (IJETCSE)* (Vol. 21).

[17]. Shah, R. K., Boruah, F., & Parween, N. (2015). Synthesis and Characterization of ZnO Nanoparticles using Leaf Extract of *Camellia sinesis* and Evaluation of their Antimicrobial Efficacy. In *Int.J.Curr.Microbiol.App.Sci* (Vol. 4, Issue 8). http://www.ijcmas.com

[18]. Li, X., Robinson, S. M., Gupta, A., Saha, K., Jiang, Z., Moyano, D. F., Sahar, A., Riley, M. A., & Rotello, V. M. (2014). Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. *ACS Nano*, *8*(10), 10682–10686. https://doi.org/10.1021/nn5042625

[19]. Yedurkar, S., Maurya, C., & Mahanwar, P. (2016). Biosynthesis of Zinc Oxide Nanoparticles Using *Ixora coccinea* Leaf Extract-A Green Approach. *Open Journal of Synthesis Theory and Applications*, *5*, 1–14. https://doi.org/10.4236/ojsta.2016.51001

[20]. Fatimah, I. (2018). Biosynthesis and characterization of ZnO nanoparticles using rice bran extract as low-cost templating agent. *Journal of Engineering Science and Technology*, *13*(2), 409–420.

[21]. Khaing, M. M., Kyaw Thu, M., Kyaw, T., Tin, T., & Lwin, T. (2018). Green Synthesis of Zinc Oxide Nanoparticles Using Tropical Plants and Their Characterizations. *International Journal of Scientific & Engineering Research*, *9*(8), 1627–1632. http://www.ijser.org

[22]. Paul, S., & Kumar Ban, D. (2014). Synthesis, characterization and the application of ZnO Nanoparticles in biotechnology. *International Journal of Advances in Chemical Engineering and Biological Sciences*, *1*(1). https://doi.org/10.15242/ijacebs.c1113015

[23]. Ahmad, Z., Ullahkha, F., Mahmood, S., & Mahmood, T. (2018). Different approaches for the synthesis of zinc oxide nanoparticles. In *Open J. Chem* (Vol. 1, Issue 1).

[24]. Hajiashrafi, S., & Motakef-Kazemi, N. (2018). Green synthesis of zinc oxide nanoparticles using parsley extract. *Nanomedicine Research Journal*, *3*(1), 44–50. https://doi.org/10.22034/NMRJ.2018.01.007

[25]. Meruvu, H., Vangalapati, M., Chaitanya Chippada, S., & Rao Bammidi, S. (2011). Synthesis and characterization of zinc oxide nanoparticles and its antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*. *Rasayan Journal of Chemistry*, *4*(1), 217–222.

[26]. Ibrahem, E. J., Yasin, Y. S., & Jasim, O. K. (2017). Antibacterial activity of zinc oxide nanoparticles against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from burn wound infections. *Cihan University-Erbil Scientific Journal*, *2017*(Special-2), 265–277. https://doi.org/10.24086/cuesj.si.2017.n2a24

[27]. Zhang, Y., Nayak, T., Hong, H., & Cai, W. (2013). Biomedical applications of zinc oxide nanomaterials. *Current Molecular Medicine*, *13*(10), 1633–1645. https://doi.org/10.2174/15665240136661311111300 58

Bonface Juma Wafula, et. al. "ZnO Nanoparticles (ZnO-NPs): Synthesis Using *Tithonia diversifolia*, Characterization and *in-vitro*Antimicrobial Bioassays." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 13(8), (2020): pp 14-21.