

## Dry Gongronema latifolium aqueous extract mediated silver nanoparticles by one-step in-situ biosynthesis for antibacterial activities

Samson O. Aisida<sup>a,b,c,d,i,\*</sup>, Kenneth Ugwu<sup>e</sup>, Assumpta C. Nwanya<sup>a,c,d</sup>, Paul A. Akpa<sup>f</sup>, I. G. Madiba<sup>c,d</sup>, A.K.H. Bashir<sup>c,d</sup>, Subelia Botha<sup>g</sup>, Paul M. Ejikeme<sup>h</sup>, Ting-kai Zhao<sup>i,j</sup>, Ishaq Ahmad<sup>b,c,d,i</sup>, M. Maaza<sup>c,d</sup>, Fabian I. Ezema<sup>a,c,d,\*</sup>

<sup>a</sup> Department of Physics and Astronomy, University of Nigeria Nsukka 410001, Nigeria

<sup>b</sup> National Centre for Physics, Quaid-i-Azam University campus, Islamabad 44000, Pakistan

<sup>c</sup> Nanosciences African Network (NANOAFNET), iThemba LABS-National Research

<sup>d</sup> UNESCO-UNISA Africa Chair in Nanosciences/Nanotechnology, College of Graduate Studies, University of South Africa (UNISA), Muckleneuk Ridge, P.O. Box 392, Pretoria, South Africa

<sup>e</sup> Department of Microbiology, University of Nigeria, Nsukka 410001, Nigeria

<sup>f</sup> Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Nigeria

<sup>g</sup> Electron Microscope Unit, University of the Western Cape, P.B X17, Bellville, South Africa

<sup>h</sup> Department of pure and industrial Chemistry, University of Nigeria, Nsukka 410001, Nigeria

<sup>i</sup> NPU-NCP Joint International Research Center on Advanced Nanomaterials and Defects Engineering, Northwestern Polytechnic University, Xi'an 710072, China

<sup>j</sup> School of Materials Science & Engineering, Northwestern Polytechnical University, Xi'an 710072, China

### ARTICLE INFO

#### Keywords:

Silver nanoparticles  
Gongronema latifolium  
biosynthesis  
antibacterial activities

### ABSTRACT

*In-situ* biosynthesis of silver nanoparticles for antibacterial activities doped with aqueous extract of dry *Gongronema Latifolium* (DGL) is presented in this work. The absorbance was determined using a UV-Visible analysis with a peak of 428 nm. The obtained particles confirmed by the TEM image analysis were spherical in shape with an average particle size between 5 and 20 nm. A single crystalline phase of FCC was confirmed by the powder. The functional groups showed the phytochemical inherent in the sample. The colloidal solution of DGL-AgNPs with MIC of 10 µg/ml showed a noteworthy functional susceptibility against the bacterial strains, particularly, *K. pneumonia* with susceptibility greater than gentamicin. Based on these observations, the formulated DGL-AgNPs show strong bactericidal against the pathogens with facile, benign, eco-friendly, biocompatibility and innocuous synthesis procedures. This can serve as an efficient bactericidal agent.

### 1. Introduction

The application of nanomaterials has received tremendous attention via interdisciplinary fields of specialization such as nanomedicine [1,2], pharmacology [3], material science [4], food technology [5], biomarkers [6] and water treatment in our environment [7]. The biomedical applications of nanotechnology have witnessed tremendous feat with special applications such as targeted drug delivery for cancer therapy [8], magnetic resonance imaging for cancer therapy [9,10], hyperthermia for cancer therapy [11,12], antibacterial activities against multi-drug resistance bacterial strains [13,14,15,16,17] etc. Metallic nanoparticles (MNPs) such as Ag, Au, Zn, Fe, Ni and their oxides serve as better and potential antibacterial and antimicrobial agents owing to

their stability and specified properties [18,19,20,21]. Among which, Ag nanoparticles (Ag-NPs) are the most potent as an alternative antibacterial and antimicrobial agent. This is owing to its low toxicity, biocompatibility, high surface to volume ratio, oxidation resistance and chemical stability [22,23,24,25,26].

To obtain the desired particle size, shape and functionality of MNPs, various methods, such as Solvothermal method [27], co-precipitation process [28], microwave-assisted [29,30], sol-gel method [31], Microemulsion [32], chemical reduction [33], Electrochemical method [34,35] condensation via evaporation [36] laser ablation [37,38], hydrothermal/thermal method [39] and Tollens process [40,41], via physical and chemical routes have been employed for the synthesis of NPs. The NPs produced by these methods via surfactants that are toxic as a

\* Corresponding authors

E-mail addresses: [samson.aisida@unn.edu.ng](mailto:samson.aisida@unn.edu.ng) (S.O. Aisida), [fabian.ezema@unn.edu.ng](mailto:fabian.ezema@unn.edu.ng) (F.I. Ezema).

<https://doi.org/10.1016/j.surfin.2021.101116>

Received 29 May 2020; Received in revised form 24 March 2021; Accepted 26 March 2021

Available online 2 April 2021

2468-0230/© 2021 Elsevier B.V. All rights reserved.

reducing and capping agent often produced NPs that are not biocompatible and eco-friendly. Owing to these effects, researchers have devised a protocol that is more biocompatible, often known as green/bio-synthesis [42,43,44,45,46,47]. This method involves synthesis protocols that use innocuous, facile, eco-friendly, cost-effective, biocompatible and biodegradable materials as a potential reducing and capping agents. It involves the use of green technology such as micro-organisms [48], biopolymers [49,50,16,51,52,53], extracts from leaves [54,14,15,17,15,16,55,56], extract from fruits and seeds of plants [57, 58,59] as a potential reducing and capping agents [13,14,17,15,55]. This approach is gaining much attention in contemporary nanotechnology [60,61,62]. Studies have shown that the adopted synthesis protocols, the interacting chemicals, calcination effect, the absorption/interfaces of the NPs with the reducing/capping agents, go a long way to influence the properties and the functionalization of the formulated nanoparticles [63,50,49].

To enhance the biocompatibility of the formulated nanoparticle, we employed a facile and biosynthesis protocol of using extract prepared from room temperature dry *Gongronema latifolium* as a potential reducing agent. The phytochemicals (PTC) inherent in DGL, as observed by many authors is bloated with flavonoid, alkaloid, Tannin and Saponin, the major bioactive compounds in all edible plants [64,65]. Owing to the intrinsic PTC in DGL, it serves as a good reducing agent for the synthesis of the formulated DGL-AgNPs, a sequel to its affinity towards Ag ions formation [66] when compared to wet *Gongronema latifolium* as we have previously reported [13].

In this work, a one-step in-situ biosynthesis co-precipitation protocol of silver nanoparticles using dry leaves extract of GL bloated in PTC as a potential reducing agent is presented. The bactericidal potency of DGL-AgNPs against *E. coli*, *S. aureus* and *K. pneumonia* were presented for the first time. It is noteworthy from the analysis that the bactericidal activities of DGL-AgNPs show strong susceptibility to the chosen pathogens with concentration-dependent compared with gentamicin (a standard drug). Based on these observations, the formulated DGL-AgNPs show strong bactericidal against the pathogens with facile, benign, eco-friendly, biocompatibility and innocuous synthesis procedures.

## 2. Materials and Methods

### 2.1. Materials

Sigma-Aldrich products of anhydrous silver nitrate ( $\text{AgNO}_3$ ) with high purity were used as procured without further purifications. Gram (+ve) *Staphylococcus aureus* (*S. aureus* ATCC 21214), Gram (-ve) *Klebsiella pneumoniae* (*K. pneumonia* ATCC 300402) and *Escherichia coli* (*E. coli* ATCC 21711) was given by the classic microbiology laboratory, University of Nigeria, Nusska. Analytical grade of Muller-Hinton agar and broth were used for the bactericidal assay. The leaves of GL were obtained in the morning from a garden in the University of Nigeria community. 1mg/mL gentamicin as +ve control and distilled water (DW) as -ve control were used.

### 2.2. Characterization Techniques

The crystallinity of the samples was determined by powder X-ray diffractometer (the Shimadzu-7000, Japan with Cu-K $\alpha$  radiation and  $\lambda = 1.5406 \text{ \AA}$ ) with scanning  $2\theta$  range of  $20^\circ$  to  $80^\circ$ . The morphology was analyzed using transmission electron microscopy (TEM) (HITACHI H-8100, Japan). Fourier transform infrared (FTIR) spectroscopy (FTIR-1650, Perkin Elmer, USA) within  $500 - 4000 \text{ cm}^{-1}$  was used to obtain the bio-conjugate functional groups. The absorbance of DGL-AgNPs in the range of 300 to 700 nm was evaluated using a UV-Visible spectrophotometer (Shimadzu Uv-7504, Japan).

### 2.3. Preparation of DGL aqueous extract

The obtained fresh leaves were washing gently with DW three times for purity. The clean GL leaves were subjected to drying at room temperature ( $T_R$ ) for 72 h. The dry GL (DGL) was turned into powder using Philip's electric blender. 10 g of the powdered GL was gently dispersed in 100 mL DW and heat to  $80^\circ \text{C}$  for 1 h while shaken intermittently. After cooling, Whitman No.1 filter paper was used to separate the extract and the residues. The filtrate is kept in the freezer below  $-4^\circ \text{C}$  for the biosynthesis.

### 2.4. In-situ biosynthesis of DGL-AgNPs

Three concentrations of the precursor  $\text{AgNO}_3$  (1, 2 and 3mM) were respectively dispersed in DW (100 mL) and stirred for 1 h at 800 rpm for homogeneity. 20 mL aqueous extract of DGL was then added to 80 mL of  $\text{AgNO}_3$  solution dropwise to make a complete 100 mL solution. This solution was stirred for another 2h. The solution was covered with aluminum foil owing to the photoluminescence of silver solution. After which the homogeneous solution is thermally treated at  $80^\circ \text{C}$  until the liquid is completely evaporated. The gel-like formed was transferred to a muffle furnace for 6 h at  $80^\circ \text{C}$ . The obtained nanoparticles were washed with ethanol followed by DW many times and annealed in a furnace at  $400^\circ \text{C}$  for 2 h. The DGL-AgNPs from the respective concentration was named a, b and c. The reaction mechanism is described in Fig.1.

### 2.5. Bactericidal activities

A modified well-diffusion method (WDM) previously used was adopted for the analysis of the bactericidal activity of the DGL-AgNPs [67]. The antibacterial assay of Ag-NPs was evaluated and tested using *E. coli*, *S. aureus* and *K. pneumonia* bacteria strain by WDM. An overnight fresh culture as inoculums of the pathogenic strains was seeded on Mueller Hinton agar plates using sterile cotton swabs. The organisms were grown overnight in peptone water. Nutrient broth agar was used to culture the organisms at  $37^\circ \text{C}$  for 24 h in an incubator with MC Farland of 0.5 standards. The autoclaved nutrient agar medium was poured into sterilized Petri dishes to form jell. The inoculum at  $\approx 10^6 \text{ CFU/mL}$  was smeared evenly on the agar plate using cotton swab stick. 7 mm diameter holes bored in the plate using sterilized cork borer were filled with DGL-AgNPs in concentrations of 10%(1.0 $\mu\text{g/mL}$ ), 30% (3.0 $\mu\text{g/mL}$ ), 50%(5.0 $\mu\text{g/mL}$ ), 80%(8.0 $\mu\text{g/mL}$ ) and 100%(10.0 $\mu\text{g/mL}$ ). The plates were incubated for 24 h at  $T_R$  to enhance its diffusion. The susceptibility via the inhibition zones (IZ) was determined and recorded in the unit of a millimeter (mm).

## 3. Results and discussion

### 3.1. Structural analysis of DGL-AgNPs

The crystal structure of all the samples, as presented in Fig. 2, gives an indication that the formulated DGL-AgNPs are crystalline in nature. The peaks observed at  $2\theta$  value and their crystallographic reflection planes are  $38.1^\circ$  (111),  $44.2^\circ$  (200),  $64.4^\circ$  (220), and  $77.3^\circ$  (311) with fcc lattice phase. Sample "c" shows the pure phase of silver without impurities. We assumed that this is due to the increase in the concentration of the  $\text{AgNO}_3$  compared with sample "a" and "b" with some biogenic phases [68]. The crystallite size of the samples was analyzed using Scherer's formula (Eq. 1) with a value of 7.2, 28.0 and 33.7 nm for samples a, b and c, respectively. The crystallite size was observed to increase with respect to the concentration of  $\text{AgNO}_3$ . Also, the peaks were more pronounced as the concentration of  $\text{AgNO}_3$  increases.

$$D = 0.9 * X - ray - wavelength / \xi \cos \theta \quad (1)$$

where  $\theta$  is the Bragg diffraction angle,  $\xi$  is the full width at half maximum

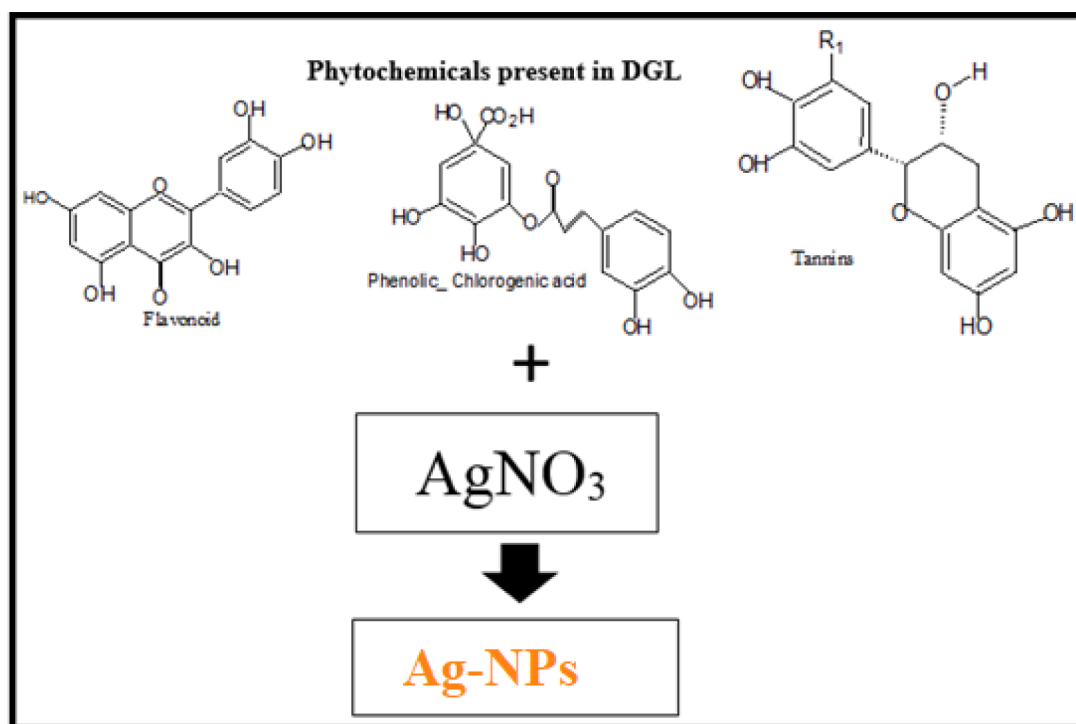


Fig. 1. Mechanism of bio-conjugate between DGL and  $\text{AgNO}_3$  formation.

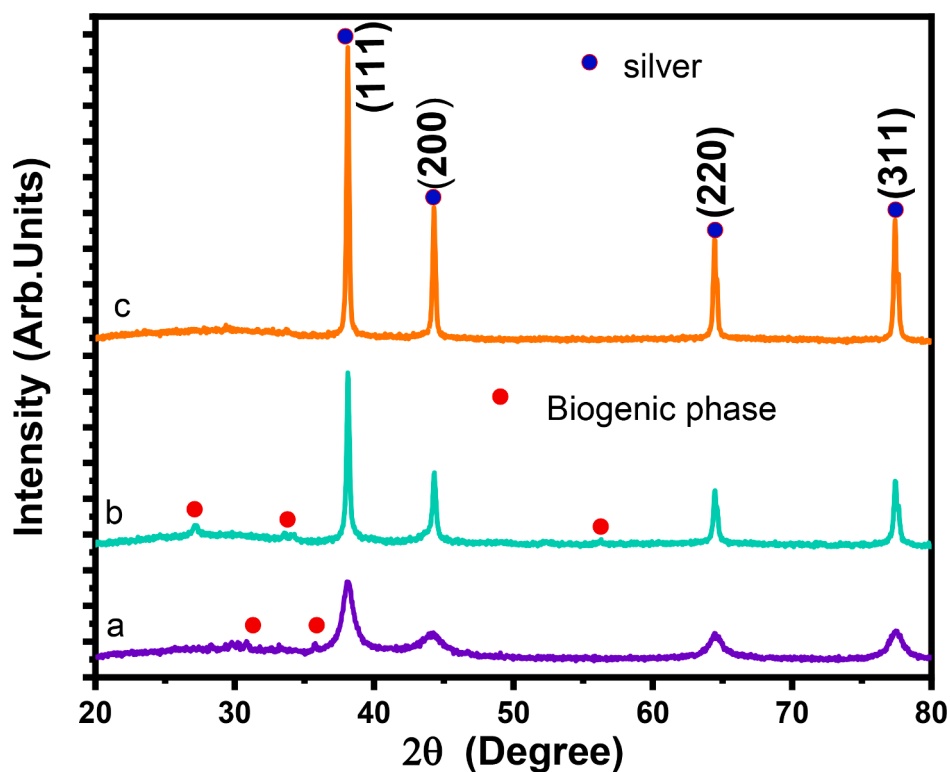


Fig. 2. XRD spectra of a, b and c.

(FWHM) in radians  $D$  is the crystallite size (nm) and the X-ray wavelength ( $\lambda$ ) = 1.5406 Å [69].

### 3.2. The HRTEM images, SAED and EDX analysis of DGL-AgNPs

The morphologies of the samples were analyzed using HRTEM

micrographs, as shown in Fig. 3 (a, b & c). Well-Dispersed particles with a narrow distribution for all the samples were observed. The results showed that the Ag-NPs nanostructured formed were spherical with a particle size ranges between 5 to 20 nm as shown in Fig. 3(g). This is in agreement with the crystallite size, as obtained by XRD. The reducing agent effectively inhibits the growth of the ripening of the DGL-AgNPs to

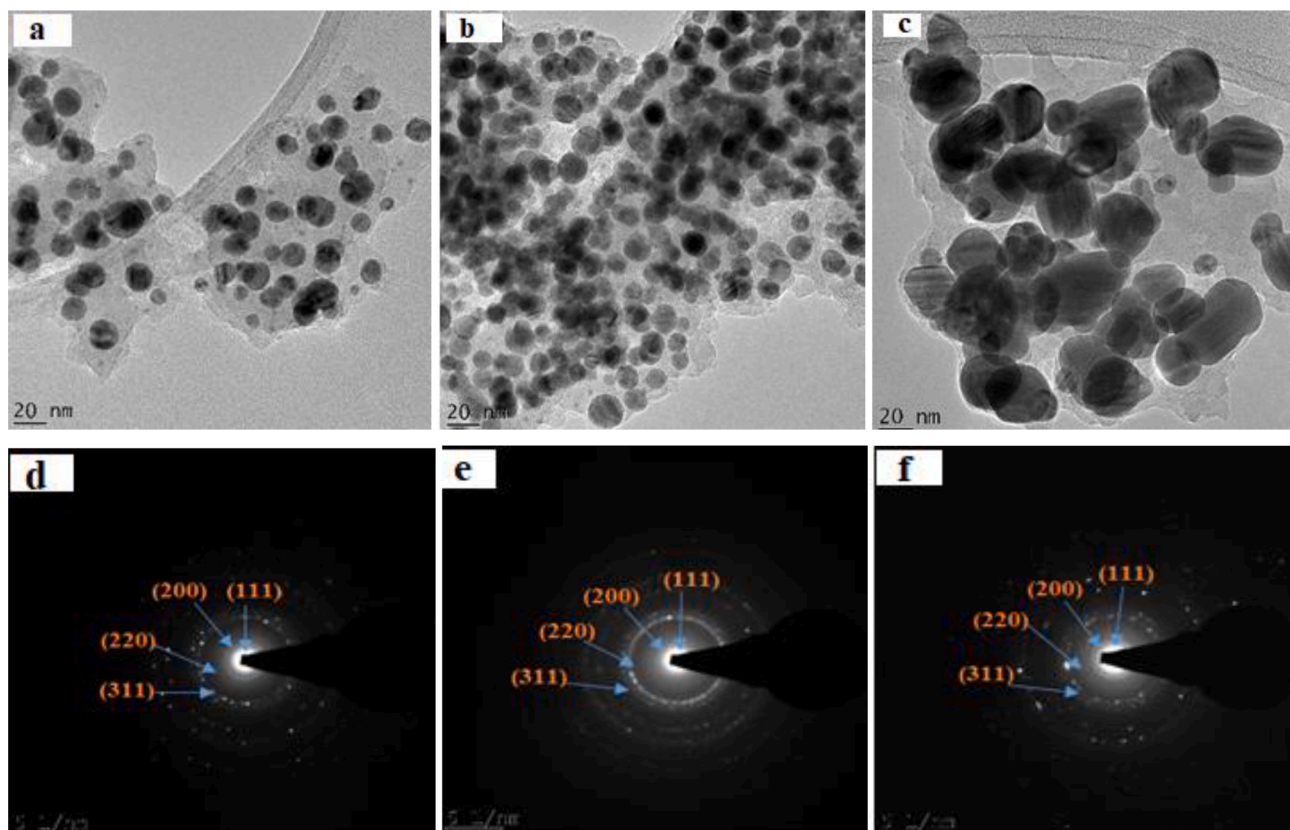


Fig. 3. (a-c) TEM images and (d-f) SAED images of samples (g) particle size distribution (h) EDX spectra analyses.

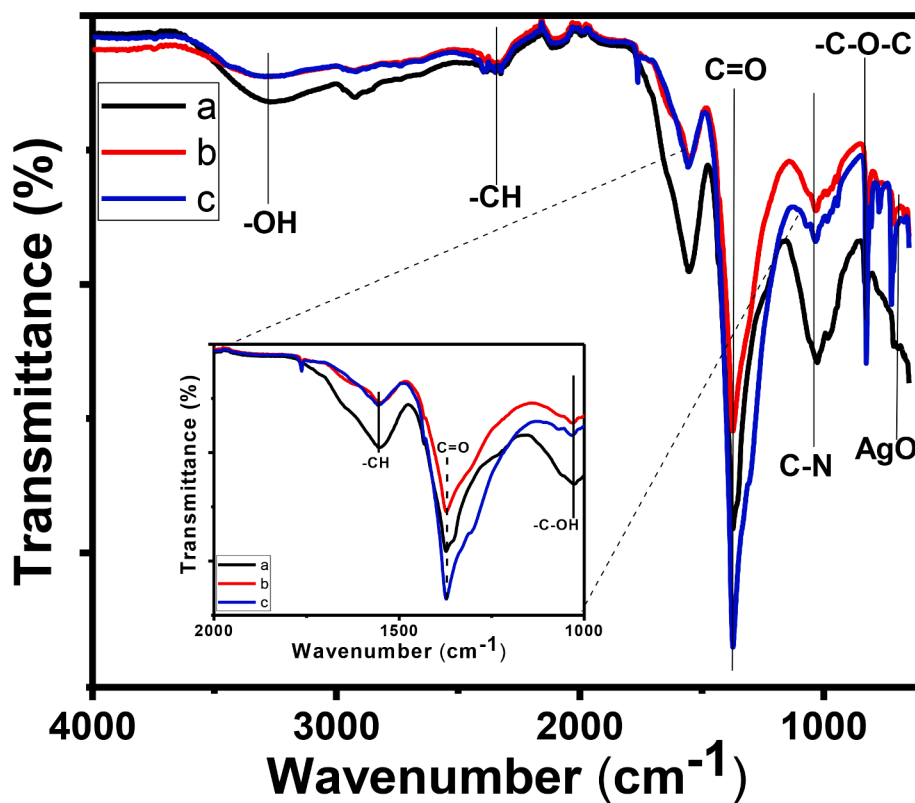


Fig. 4. FT-IR spectra of the samples.

avoid agglomeration. Also, the elemental compositions, as shown in Fig. 3 (d, e & f) were determined by SAED. The pattern of the sharp rings confirmed the polycrystallinity of the formulated DGL-AgNPs. The formed rings from (1 1 1), (2 0 0), (2 2 0) and (3 1 1) is linked to the diffraction plane of FCC. The EDX analysis, as presented in Fig. 3 (h) revealed the elemental composition of the sample and all the constituent's elements in the compound with a strong peak for Ag at 8.0 keV.

### 3.3. FTIR Spectra of DGL-AgNPs

The functional groups between the chemical compositions present in the formulated DGL-AgNPs to ascertain the PTC inherent in the samples were analyzed by FTIR spectroscopy and presented in Fig. 4. The spectra revealed the vibration bands in the samples in the wavelength range between 500 and 4000  $\text{cm}^{-1}$ . We observed that all the samples exhibited the same peaks. The peaks at 3279  $\text{cm}^{-1}$  and 2334  $\text{cm}^{-1}$  are owing to the (-OH stretching) and (-CH stretching, alkanes), respectively of the hydrocarbons group of water molecules [70,71]. The observed peak at 1349  $\text{cm}^{-1}$  is due to vibration C=O (carbonyl and esters bonded conjugate) [72,73]. The peak at 1011  $\text{cm}^{-1}$  is attributed to C-N aromatic amines. The peak at 822  $\text{cm}^{-1}$  gives the stretching vibration of C-O-C. The observed functional groups in DGL-AgNPs showed the bio-conjugate interaction between the DGL and  $\text{AgNO}_3$ ; owing to the DGL bioactive compounds towards the  $\text{Ag}^+$  to donate free electrons, with a transition from  $\text{Ag}^+$  to  $\text{Ag}^0$ . Moreover, the peak at 683  $\text{cm}^{-1}$  and below is ascribed to Ag-O surface bond in the formation of DGL-AgNPs [74,75]. These results agreed with the previous results and showed the potency of the aqueous extract as a potential reducing agent in the formulation of DGL-AgNPs [60,13,14,17].

### 3.4. UV-Visible spectra of DGL-AgNPs

The absorption spectra of DGL-AgNPs were analyzed using a UV-Vis spectrophotometer, which is presented in Fig. 5. The formation of DGL-AgNPs and the observed color change is owing to the reduction of  $\text{Ag}^+$  to DGL-AgNPs by the bioactive components in DGL [73]. A color change was observed after 1 min. with a surface plasmon resonance peak of 428 nm for the samples with increasing intensity as the concentration increases [76]. These results confirmed the formulation of DGL-AgNPs since the surface plasmon resonance peak is within  $420 \leq x \leq 470 \text{nm}$  [77,26]. This is in agreement with other research work [78,25].

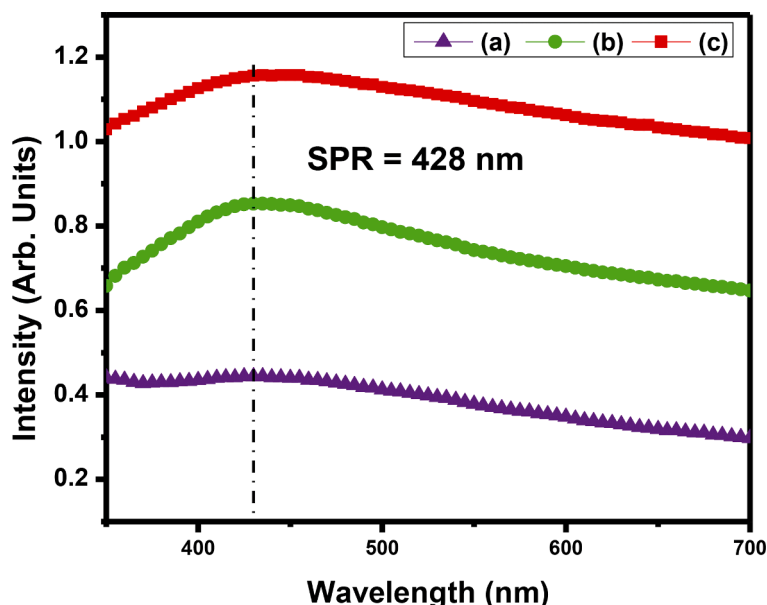


Fig. 5. UV-Visible spectra of the samples.

### 3.5. Antibacterial activity of DGL-AgNPs

The IZ of DGL-AgNPs against the selected bacterial strains measured in mm for each organism is presented as shown in Table 1. The formulated DGL-AgNPs were observed to show significant bactericidal activity towards the pathogenic organisms by inhibiting the growth of the bacterial strains, as presented in Fig. 6. The antibacterial activities of DGL-AgNPs against the bacterial strains were observed to be viable compared with Gentamicin; particularly, in *K. pneumonia*. The antibacterial activities of DGL-AgNPs with MIC of 10  $\mu\text{g}/\text{ml}$  are concentration dose-dependent i.e., it increases with the concentration dose [79,54,26], as presented in Fig. 7. Based on the susceptibility of DGL-AgNPs against the pathogenic strains, the obtained DGL-AgNPs can serve as a potential and efficient antibacterial agent owing to its activities towards the pathogens.

## 4. Conclusion

A facile, cost-effective, nontoxic and ecofriendly one step in situ process was used to prepare DGL-AgNPs via DGL to define the properties of the formulated nanoparticles and their antibacterial potency against Gram-positive and Gram-negative were reported in work. DGL was used as a fuel owing to its intrinsic PTC. The TEM analyses formed were spherical, with the particle size, ranges from 5 - 20 nm. A single phase of well crystalline samples was observed with the XRD analysis. The functional groups present in the reducing agent have been discovered from the FTIR results. This showed that DGL molecules adsorbed on the surfaces of the NPs as a barrier to prevent them from agglomeration. Also, the absorbance by UV-Vis gave a distinct SPR at 428 nm for all the samples, which is evidence of the complete absorption of DGL by

Table 1

Inhibition zone of DGL-AgNPs and gentamicin against the pathogens.

Organism	DW	Inhibition zone (mm) (MIC = 10 $\mu\text{g}/\text{ml}$ )					
		C1	C2	C3	C4	C5	Gen.
<i>S. aureus</i>	0.00	21	24	25	26	24	35
<i>K. pneumonia</i>	0.00	22	20	23	23	30	27
<i>E. coli</i>	0.00	19	22	24	25	25	34

C1 = 10  $\mu\text{g}/\text{mL}$ , C2 = 30  $\mu\text{g}/\text{mL}$ , C3 = 50  $\mu\text{g}/\text{mL}$ , C4 = 80  $\mu\text{g}/\text{mL}$  and C5 = 100  $\mu\text{g}/\text{mL}$ ,

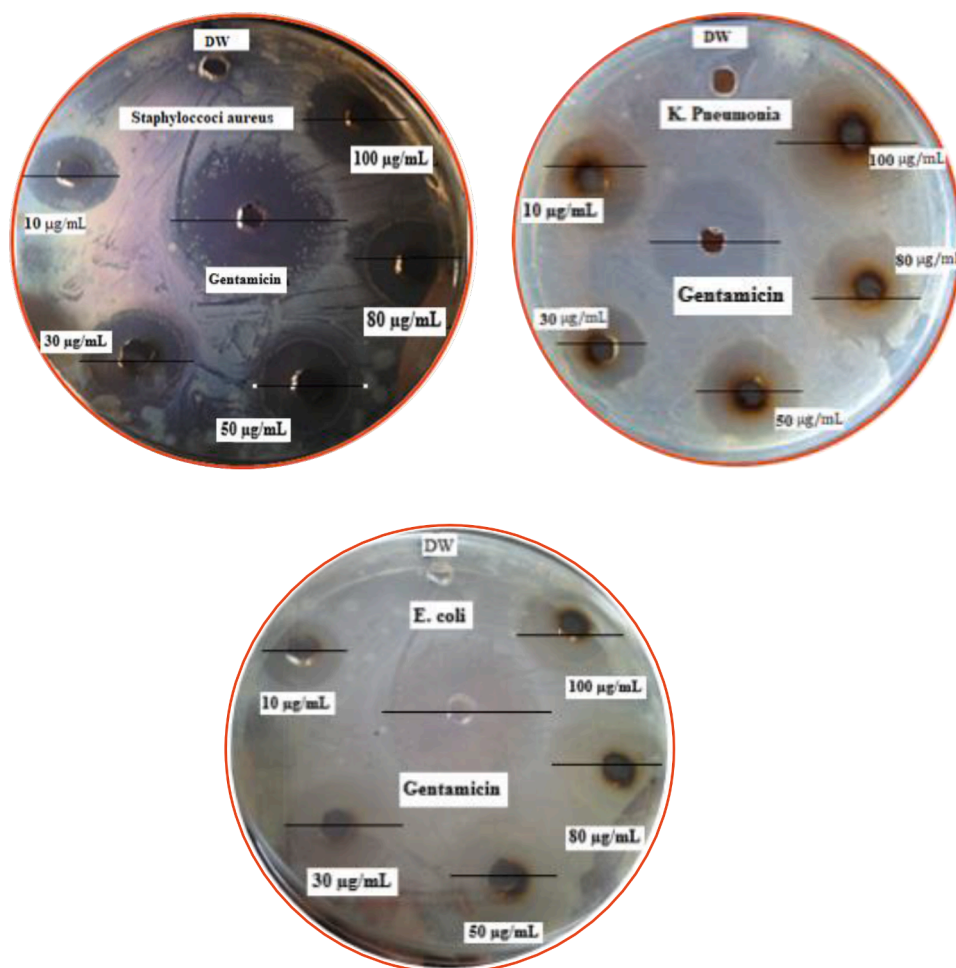


Fig. 6. The images of the zone of inhibition of DGL-AgNPs against the pathogenic strains.

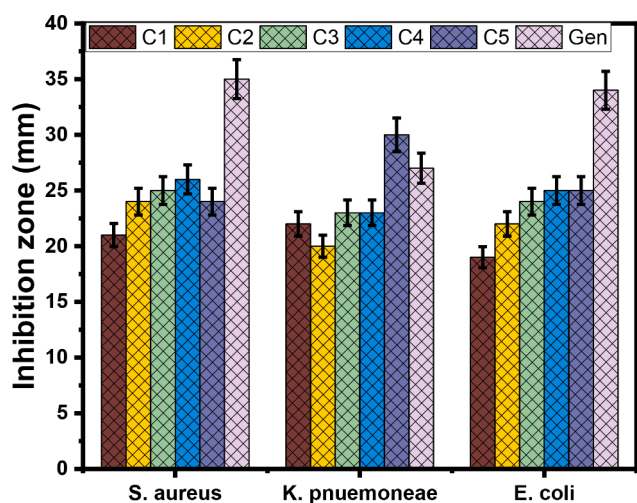


Fig. 7. Activities of DGL-AgNPs against the pathogenic strains.

AgNO<sub>3</sub>. The viability of DGL-AgNPs, was examined against three most debilitating bacterial strains: E. coli, S. aureus and K. pneumonia. It is noteworthy from the analysis that the bactericidal activities of DGL-AgNPs exhibit strong susceptibility to the chosen pathogens. Owing to these observations, the formulated DGL-AgNPs stand as a potential bactericidal agent due to its facile, benign, biocompatibility, eco-friendly and innocuous synthesis protocol.

**Credit authors statement**

**Samson O. Aisida:** Conceptualization, Data curation, Writing, Methodology, Original draft preparation, Software, Reviewing and Editing. **Kenneth Ugwu:** Visualization, Data curation **Assumpta C. Nwanya:** Visualization, Data curation **Paul A. Akpa:** Visualization, Data curation, I.G. **Madiba:** Visualization, A.K.H. **Bashir:** Visualization, **Subelia Botha:** Visualization, Data curation, **Paul M. Ejikeme:** Visualization, **Ishaq Ahmad:** Visualization, Editing **Ting-kai Zhao:** Visualization, Data curation **Malik Maaza:** Visualization, Data curation **Fabian I. Ezema:** Visualization, Data curation, Investigation, Reviewing and Editing.

**Declaration of Competing Interest**

The authors declare that they have no conflicts of interest

**Acknowledgments**

Samson O. Aisida acknowledges the NCP-TWAS Postdoc Fellowship award (NCP-CAAD/TWAS\_Fellow8408).

Fabian I. Ezema acknowledges VRSP/UNISA/90407830 Fellowship award; he also acknowledges the grant by TETFUND under contract number TETF/DESS/UNN/NSUKKA/STI/VOL.1/B4.33. Also, we thank Engr. Emeka Okwuosa for the sponsorship of 2014, 2016 and 2018 nano-conferences/workshops.

## References

- [1] A. Aliosmanoglu, I. Basaran, Nanotechnology in cancer treatment, *J. Nanomedicine Biotherapeutic Discovery*. 21 (2012) 1–3.
- [2] K. Venugopal, H.A. Rather, K. Rajagopal, M.P. Shanthy, K. Sheriff, M. Illiyas, R. A. Rather, E. Manikandan, S. Uvarajan, M. Bhaskar, M. Maaza, Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of *Syzygium aromaticum*, *Journal of Photochemistry & Photobiology, B: Biology* 167 (2017) 282–289.
- [3] K. Palaniselvam, M. Mashitah, P. Gaanty, G. Natanamurugaraj, Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report, *Saudi, Pharm. Journal* 24 (2015) 473–484.
- [4] J.M. Costa-Fernandez, R. Pereiro, A. Sanz-Medel, The Use of Luminescent Quantum Dots for Optical Sensing, *Trends Anal. Chem.* 25 (2006) 207–218.
- [5] T. Duncan, Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors, *J. Colloid Interface Sci.* 363 (2011) 1–24.
- [6] A. Schrand, L. Braydich-Stolle, J. Schlager, L. Dai, S. Hussain, Can silver nanoparticles be useful as potential biological labels? *Nanotechnology* 19 (2008), 235104.
- [7] J. Fabrega, S.N. Luoma, C.R. Tyler, T.S. Galloway, J.R. Lead, Silver nanoparticles: Behaviour and effects in the aquatic environment, *Behav. Eff. Aquat. Environ. Int.* 37 (2011) 517–531.
- [8] M. Dadashpour, A. Firouzi-Amandi, M. Pourhassan-Moghaddam, M.J. Maleki, N. Soozangar, F. Jeddi, M. Nouri, Y.P.-S. Zarghami, Biomimetic synthesis of silver nanoparticles using *Matricaria chamomilla* extract and their potential anticancer activity against human lung cancer cells, *Mater. Sci. Eng. C* 92 (2018) 902–912.
- [9] G.-P. Yan, L. Robinson, P. Hogg, Magnetic resonance imaging contrast agents: overview and perspectives, *Radiography* 13 (2007) e5–e19.
- [10] H. Zhang, L. Li, X.L. Liu, J. Jiao, C.T. Ng, J.B. Yi, Y.E. Luo, B.-H. Bay, L.Y. Zhao, M. L. Peng, N. Gu, H.M. Fan, Ultrasmall ferrite nanoparticles synthesized via dynamic simultaneous thermal decomposition for high-performance and multifunctional T1 magnetic resonance imaging contrast agent, *ACS Nano* 11 (2017) 3614–3631.
- [11] I. Sharifi, H. Shokrollahi, S. Amiri, Ferrite-based magnetic nanofluids used in hyperthermia applications, *J. Magn. Magn. Mater.* 324 (2012) 903–915.
- [12] S.A. Shah, M.U. Hashmi, S. Alam, A. Shamim, Magnetic and bioactivity evaluation of ferrimagnetic ZnFe<sub>2</sub>O<sub>4</sub> containing glass ceramics for the hyperthermia treatment of cancer, *J. Magn. Magn. Mater.* 322 (2010) 375–381.
- [13] S.O. Aisida, K. Ugwu, P.A. Akpa, A.C. Nwanya, P.M. Ejikeme, S.S. Botha, I. Ahmad, M. Maaza, F.I. Ezema, Biogenic synthesis and antibacterial activity of controlled silver nanoparticles using an extract of *Gnongronema Latifolium*, *Materials Chemistry and Physics* 237 (2019), 121859 b.
- [14] S.O. Aisida, K. Ugwu, P.A. Akpa, A.C. Nwanya, U. Nwankwo, S.S. Botha, P. M. Ejikeme, I. Ahmad, M. Maaza, F.I. Ezema, Biosynthesis of silver nanoparticles using bitter leave (*Veronica amygdalina*) for antibacterial activities, *Surfaces and Interfaces* 17 (2019), 100359 a.
- [15] S.O. Aisida, N. Madubuonu, M.H. Alnasir, I. Ahmad, S. Botha, M. Maaza, F. I. Ezema, Biogenic synthesis of iron oxide nanorods using *Moringa oleifera* leaf extract for antibacterial applications, *Appl Nanosci* 10 (2020) 305–315.
- [16] S.O. Aisida, E. Ugwoke, A. Uwais, C. Iroegbu, S. Botha, I. Ahmad, M. Maaza, F. I. Ezema, Incubation period induced biogenic synthesis of PEG enhanced *Moringa oleifera* silver nanocapsules and its antibacterial activity, *J Polym Res* 26 (2019) 225.
- [17] N. Madubuonu, S.O. Aisida, A. Ali, I. Ahmad, Z. Ting-kai, S. Botha, M. Maaza, F. I. Ezema, Biosynthesis of iron oxide nanoparticles via a composite of *Psidium guajava*-*Moringa oleifera* and their antibacterial and photocatalytic study, *Journal of Photochemistry and Photobiology B: Biology* 199 (2019), 111601.
- [18] I. Sondi, S.B. Sondi, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram negative bacteria, *J. Colloid Interface Sci.* 275 (2004) 177–182.
- [19] S.O. Aisida, K. Ugwu, P. Akpan, A.C. Nwanya, A.K.H. Bashir, I. Madiba, N. U. Nwankwo, I. Ahmed, F.I. Ezema., Synthesis and characterization of iron oxide nanoparticles capped with *Moringa Oleifera*: The mechanisms of formation effects on the optical, structural, magnetic and morphological properties, in: *Material today: Proceeding* 214-218, 2021, p. 36. Vols.
- [20] S.O. Aisida, M.H. Alnasir, S. Botha, A.K.H. Bashir, R. Bucher, I. Ahmad, T.-k. Zhao, M. Maaza, F.I. Ezema, The role of polyethylene glycol on the microstructural, magnetic and specific absorption rate in thermoablation properties of Mn-Zn ferrite nanoparticles by sol-gel protocol, *European polymer journal* 132 (2020), 109739.
- [21] S.O. Aisida, A. Batool, F.M. Khan, L. Rahman, A. Mahmood, I. Ahmad, T.-k. Zhao, M. Maaza, F.I. Ezema, Calcination induced PEG-Ni-ZnO nanorod composite and its biomedical applications, *Materials Chemistry and Physics* 255 (2020), 123603.
- [22] K. Gudikandula, V. Pranitha, M.C. Singara, Biogenic synthesis of silver nanoparticles from white rot fungi: Their characterization and antibacterial studies, *Open nano* 17 (2017).
- [23] A. Ravindran, C. Preethy, S.S. Khan, Biofunctionalized silver nanoparticles: advances and prospects, *Colloids Surf. B: Biointerfaces* 105 (2013) 342–352.
- [24] N. R., M. S., J. P. S., P. P., Green synthesis and characterization of bioinspired silver, gold and platinum nanoparticles and evaluation of their synergistic antibacterial activity after combining with different classes of antibiotics, *Materials Science and Engineering: C* 96 (2019) 693–707.
- [25] S.O. Aisida, K. Ugwu, P. Akpan, A.C. Nwanya, P.M. Ejikeme, S. Botha, I. Ahmed, F. I. Ezema, Morphological, optical and antibacterial study of green synthesized silver nanoparticles via *Vernonia amygdalina*, *Material today: proceedings* 36 (2021) 199–203.
- [26] S.O. Aisida, K. Ugwu, A.C. Nwanya, A.K.H. Bashir, N.U. Nwankwo, I. Ahmed, F. I. Ezema., Biosynthesis of silver oxide nanoparticles using leave extract of *Telfairia Occidentalis* and its antibacterial activity, *Material today: Proceedings* 36 (2021) 208–213.
- [27] B. Mahtlig, E. Gutmann, M. Reibold, D. Meyer, H. Botcher, Synthesis of Ag and Ag/SiO<sub>2</sub> sols by solvothermal method and their bactericidal activity, *J Sol-Gel Sci Technol* 51 (2009) 204–214.
- [28] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, E.L. Vander, R. Muller, Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications, *Chem. Rev.* 108 (2008), 2064–21.
- [29] J. Chen, K. Wang, J. Xin, Y. Jin, Microwave-assisted green synthesis of silver nanoparticles by carboxymethyl cellulose sodium and silver nitrate, *Mater Chem Phys* 108 (2008) 421–424.
- [30] F.N. Sayed, V. Polshettiwar, Facile and Sustainable Synthesis of Shaped Iron Oxide Nanoparticles: Effect of Iron Precursor Salts on the Shapes of Iron Oxides, *Sci. Rep.* 5 (2015) 9733.
- [31] S.A. Dharamvir, K. Rekha, Y. Rachna, Y. Indu, Synthesis and Characterization of Sol-Gel Prepared Silver Nanoparticles, *International Journal of Nanoscience* 13 (2014), 1450004.
- [32] P. Cozzoli, R. Comparelli, E. Fanizza, M. Curri, A. Agostiano, D. Laub, Photocatalytic synthesis of silver nanoparticles stabilized by TiO<sub>2</sub> nanorods: A semiconductor/metal nanocomposite in homogeneous nonpolar solution, *J Am Chem Soc* 126 (2004) 3868–3879.
- [33] M. Oliveira, D. Ugarte, D. Zanchet, A. Zarbin, Influence of synthetic parameters on the size, structure, and stability of dodecanethiol-stabilized silver nanoparticles, *J Colloid Interface Sci* 292 (2005) 429–435.
- [34] H. Ma, B. Yin, S. Wang, Y. Jiao, W. Pan, S. Huang, S. Chen, F. Meng, Synthesis of silver and gold nanoparticles by a novel electrochemical method, *Chem Phys Chem* 24 (2004) 68–75.
- [35] Y. Zhang, F. Chen, J. Zhuang, Y. Tang, D. Wang, Y. Wang, A. Donga, N. Ren, Synthesis of silver nanoparticles via electrochemical reduction on compact zeolite film modified electrodes, *Chem Commun* 24 (2002) 2814–2815.
- [36] F. Krus, H. Fissan, B. Rellinghaus, Sintering and evaporation characteristics of gas-phase synthesis of size-selected PbS nanoparticles, *Mater Sci Eng B* 69 (2000) 329–334.
- [37] F. Mafune, J. Kohno, Y. Takeda, T. Kondow, H. Sawabe, Structure and stability of silver nanoparticles in aqueous solution produced by laser ablation, *J Phys Chem B* 104 (2000) 8333–8337.
- [38] J. Sylvestre, A. Kabashin, E. Sacher, M. Meunier, J. Luong, Stabilization and size control of gold nanoparticles during laser ablation in aqueous cyclodextrins, *J Am Chem Soc* 126 (2004) 7176–7177.
- [39] M. Khalil, J. Yu, N. Liu, R.L. Lee, Hydrothermal Synthesis, Characterization, and Growth Mechanism of Hematite Nanoparticles, *J. Nanopart. Res.* 16 (2014) 2362.
- [40] J. Soukupova, L. Kvitek, A. Panacek, T. Nevecna, R. Zboril, Comprehensive study on surfactant role on silver nanoparticles (NPs) prepared via modified Tollens process, *Mater Chem Phys* 111 (2008) 77–81.
- [41] Y. Yin, Z.-Y. Li, Z. Zhong, B. Gates, S. Venkateswaran, Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process, *J Mater Chem* 12 (2002) 522–527.
- [42] M. Mosaviniya, T. Kikhavani, M. Tanzifi, M.T. Yarak, P. Tajbakhsh, A. Lajevardi, Facile green synthesis of silver nanoparticles using *Crocus Haussknechtii* Bois bulb extract: Catalytic activity and antibacterial properties, *Colloid and Interface Science Communications* 33 (2019), 100211.
- [43] J.R. Nakkala, R. Mata, K. Raja, V.K. Chandra, S.R. Sadras, Green synthesized silver nanoparticles: Catalytic dye degradation, in vitro anticancer activity and in vivo toxicity in rats, *Materials Science and Engineering: C* 91 (2018) 372–381.
- [44] M. Behravan, A.H. Panahi, A. Naghizadeh, M. Ziaee, R. Mahdavi, A. Mirzapour, Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity, *International Journal of Biological Macromolecules* 124 (2019) 148–154.
- [45] O.T. Jemilugba, E.H.M. Sakho, S. Parani, V. Mavumengwana, O.S. Oluwafemi, Green synthesis of silver nanoparticles using *Combretum erythrophyllum* leaves and its antibacterial activities, *Colloid and Interface Science Communications* 31 (2019), 100191.
- [46] R.G. Saratale, I. Karuppusamy, G.D. Saratale, A. Pugazhendhi, G. Kumar, Y. Park, G.S. Ghodake, R.N. Bhargava, J.R. Banu, H.S. Shin, A comprehensive review on green nanomaterials using biological systems: recent perception and their future applications, *Colloids Surf. B: Biointerfaces* 170 (2018) 20–35.
- [47] W.R. Rolim, M.T. Pelegrino, B. d. A. Lima, L.S. Ferraz, F. N.Costa, J.S. Bernardes, T. Rodrigues, M. Brocchi, A.B. Seabra, Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity, *Applied Surface Science* 463 (2019) 66–74.
- [48] P. Shankar, S. Shobana, I. Karuppusamy, A. Pugazhendhi, V.S. Ramkumar, S. Arvindnarayan, G. Kumar, A review on the biosynthesis of metallic nanoparticles (gold and silver) using bio-components of microalgae: formation mechanism and applications, *Enzym. Microb. Technol.* 95 (2016) 28–44.
- [49] S.O. Aisida, P.A. Akpa, I. Ahmad, M. Maaza, F.I. Ezema, Influence of PVA, PVP and PEG doping on the optical, structural, morphological and magnetic properties of zinc ferrite nanoparticles produced by thermal method, *Physica B: Condensed Matter* 571 (2019) 130–136, a.
- [50] S.O. Aisida, I. Ahmad, F.I. Ezema, Effect of calcination on the microstructural and magnetic properties of PVA, PVP and PEG assisted zinc ferrite nanoparticles, *Physica B: Condensed Matter* (2019).
- [51] S.O. Aisida, P.A. Akpa, I. Ahmad, T. Zhao, M. Maaza, F.I. Ezema, Bio-inspired encapsulation and functionalization of iron oxide nanoparticles for biomedical applications, *European polymer journal* (2019).

- [52] B. Bharat, K. Vitaly, K. Boris, K. Nikolai, P. Gopinath, Impact of albumin based approaches in nanomedicine: Imaging, targeting and drug delivery, *Advances in Colloid and Interface Science* 246 (2017) 13–39.
- [53] D.-l. Su, P.-j. Li, M. Ning, G.-y. Li, Y. Shan, Microwave assisted green synthesis of pectin based silver nanoparticles and their antibacterial and antifungal activities, *Materials Letters* 244 (2019) 35–38.
- [54] S.O. Aisida, K. Ugwu, P.A. Akpa, A.C. Nwanaya, P.M. Ejikeme, S.S. Botha, I. Ahmad, M. Maaza, F.I. Ezema, Biogenic synthesis and antibacterial activity of controlled silver nanoparticles using an extract of *Gongronema Latifolium*, *Materials Chemistry and Physics* 237 (2019), 121859 b.
- [55] R. Tahir, B. Muhammad, M.I. Hafiz, L. Chuanlong, Green biosynthesis of silver nanoparticles using leaves extract of *Artemisia vulgaris* and their potential biomedical applications, *Colloids and Surfaces B: Biointerfaces* 158 (2017) 408–415.
- [56] S.V. Ravichandran, S. Sivadasan, A.A.S. Syed, H. Rajak, Green synthesis of silver nanoparticles using *Atrocarpus altilis* leaf extract and the study of their antimicrobial and antioxidant activity, *Mater. Lett.* 180 (2016) 264–267.
- [57] V. Dhand, L. Soumya, S. Bharadwaj, S. Chakra, D. Bhatt, B. Sreedhar, Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activity, *Mater. Sci. Eng. C* 58 (2016) 36–43.
- [58] F.K. Alsammarraie, W. Wang, P. Zhou, A. Mustapha, M. Lin, Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities, *Colloids Surf. B: Biointerfaces* 171 (2018) 398–405.
- [59] M. Mosaviniya, T. Kikhavani, M. Tanzifi, M.T. Yaraki, P. Tajbakhsh, A. Lajevardi, Facile green synthesis of silver nanoparticles using *Crocus Haussknechtii* Bois bulb extract: Catalytic activity and antibacterial properties, *Colloid and Interface Science Communications* 33 (2019), 100211.
- [60] R.R. Wallace, T.P. Milena, d.A.L. Bruna, S.F. Leticia, N.C. Fanny, S.B. Juliana, R. Tiago, B. Marcelo, B.S. Amedea, Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity, *Applied Surface Science* 463 (2019) 66–74.
- [61] S.J. Mane, G.H. Nikam, R.S. Dhabbe, S.R. Sabale, B.V. Tamhankar, G.N. Mulik, Green synthesis of silver nanoparticles by using carambola fruit extract and their antibacterial activity, *Adv. Nat. Sci.: Nanosci. Nanotechnol.* 6 (2015), 045015.
- [62] R. Iman, G. Maryam, B. Saeed, Biosynthesis of silver nanoparticles using leaf extract of *Satureja hortensis* treated with NaCl and its antibacterial properties, *Microporous and Mesoporous Materials* 264 (2018) 240–247.
- [63] B. Wiley, Y.G. Sun, B. Mayers, Y.N. Xia, Shape-controlled synthesis of metal nanostructures: the case of silver, *Chem. – Eur. J.* 11 (2005) 454–463.
- [64] H.P. Chhetri, N. Vogel, S. J. K. Anupa, e.a. Mansoor S, Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal, *Kathmandu University Journal of Science, Engineering and Technology* 1 (2008) 49–54.
- [65] I. Chinedu, O.U. Friday, Phytochemical Analysis of *Gongronema latifolium* Benth Leaf Using Gas Chromatographic Flame Ionization Detector, *International Journal of Chemical and Biomolecular Science* 1 (2015) 60–68.
- [66] W. Jian, L. Zhang, K.-C. Siu, A. Song, J.-Y. Wu, Formation and Physicochemical Properties of Silver Nanoparticles with Various Exopolysaccharides of a Medicinal Fungus in Aqueous Solution, *Molecules* 22 (2016) 50.
- [67] G. Manjul, J. n. P. Geeta, Synthesis and Catalytic and Biological Activities of Silver and Copper Nanoparticles Using *Cassia occidentalis*, *International Journal of Biomaterials* 2018 (2018).
- [68] B. Devaraj, M.J. Diviya, V. Seerangaraj, V. Bhuvaneshwari, Biosynthesis of silver nanoparticles using stem bark extracts of *Diospyros montana* and their antioxidant and antibacterial activities, *Journal of Nanostructure in chemistry* 8 (2018) 83–92.
- [69] B. Cullity, *Element of X-ray Diffraction*, 2nd ed, Addison-Wesley, London, 1978, p. 102, ed.p. p.
- [70] V. Ahluwalia, S. Elumalai, V. Kumar, S. Kumar, R. Sangwan, Nano silver particle synthesis using *Swertia paniculata* herbal extract and its antimicrobial activity, *Microb. Pathog.* 114 (2018) 402–408.
- [71] R. Rivera-Rangel, M. González-Munhoz, M. Avila-Rodriguez, T. Razo-Lazcano, C. Solans, Green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using geranium leaf aqueous extract as a reducing agent, *Colloids Surf. A* 536 (2018) 60–67.
- [72] Q. Sun, X. Cai, J. Li, M. Zheng, Z. Chen, C. Yu, Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity, *Colloids Surf. A Physicochem. Eng. Aspects* 444 (2014) 226–231.
- [73] T. Carballo, M. Gil, X. Gómez, F. González-Andrés, A. Morán, Characterization of different compost extracts using Fourier-transform infrared spectroscopy (FTIR) and thermal analysis, *Biodegradation* 19 (2008) 815–830.
- [74] K. Kombariah, J.V. Judith, L.K. John, M. Bououdina, R.R. Jothi, A. A.-I. Hamad, Okra extract-assisted green synthesis of  $\text{CoFe}_2\text{O}_4$  nanoparticles and their optical, magnetic and antimicrobial properties, *Material chemistry and Physics* 204 (2018) 410–419.
- [75] C. Ladole, Preparation and characterization of spinel zinc ferrite  $\text{ZnFe}_2\text{O}_4$ , *Int. J. Chem. Sci.* 10 (2012) 1230.
- [76] B. Moldovan, L. David, M. Achim, S. Clichici, G.A. Filip, A green approach to phytomediated synthesis of silver nanoparticles using, *Journal of Molecular Liquids* (2016) 271–278.
- [77] M.A. Jalaluddin, A.A. Mohammad, M.K. Haris, A.A. Mohammad, C. Inho, Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques, *Sci Rep* 6 (2016) 20414.
- [78] B. Mahmoodreza, H. Ayat, N. Ali, Z. Masood, M. Roya, M. Aliyar, Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity, *Int. J. Biol. Macromol.* 124 (2019) 148–154.
- [79] M. Valodkar, S. Modi, A. Pal, S. Takore, Synthesis and anti-bacterial activity of Cu, Ag and Cu-Ag alloy nanoparticles: a green approach, *Materials Research Bulletin* 46 (2011) 384–389.