Research highlight

Agbaje Lateef*, Monsurat A. Akande, Musibau A. Azeez, Sunday A. Ojo, Bolaji I. Folarin, Evariste B. Gueguim-Kana and Lorika S. Beukes

Phytosynthesis of silver nanoparticles (AgNPs) using miracle fruit plant (*Synsepalum dulcificum*) for antimicrobial, catalytic, anticoagulant, and thrombolytic applications

DOI 10.1515/ntrev-2016-0039
Received May 28, 2016; accepted June 21, 2016; previously published online September 24, 2016

Abstract: In the present work, we report the phytosynthesis of AgNPs mediated by leaf and seed extracts of *Synsepalum dulcificum*. The extracts catalyzed the formation of brown colloidal AgNPs, which stabilized in 10 min. The leaf and seed AgNPs yielded surface plasmon resonance at 440 and 438.5 nm, respectively. Prominent peaks at 3408, 2357, 2089, and 1639 cm^{-1} were recorded for leaf AgNPs, whereas 3404, 2368, 2081, and 1641 cm^{-1} were revealed for seed-mediated AgNPs from Fourier transform infrared data. These showed the involvement of phenolic compounds and proteins in the phytosynthesis. The particles were fairly spherical and crystalline in nature having size of 4–26 nm, with prominence of silver in the colloidal solutions. The particles inhibited the growth of drug-resistant strains of *Pseudomonas aeruginosa* and *Klebsiella granulomatis* with zone of inhibition of 11–24 mm. Also, the phytosynthesized AgNPs completely inhibited the growth of *Aspergillus flavus* and *Aspergillus niger*. In addition, by using 20 μg/ml of AgNPs, malachite green was degraded by approximately 80% in 24 h. Similarly, the particles displayed blood anticoagulant activities as well as achieved thrombolysis. The AgNPs can be explored for biomedical and catalytic applications. The report is the first on the eco-friendly synthesis of nanoparticles by *S. dulcificum*.

Keywords: antimicrobial activity; phytosynthesis; silver nanoparticles; *Synsepalum dulcificum*; thrombolysis.

***Corresponding author: Agbaje Lateef,** Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria; and Nanotechnology Research Group (NANO$^+$), Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria, Tel.: +234 8037400520, e-mail: alateef@lautech.edu.ng; agbaje72@yahoo.com
Monsurat A. Akande, Sunday A. Ojo and Bolaji I. Folarin: Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria
Musibau A. Azeez: Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria; and Nanotechnology Research Group (NANO$^+$), Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria
Evariste B. Gueguim-Kana: Department of Microbiology, University of KwaZulu-Natal, Private Bag X01, Scottsville, PieterMaritzburg 3209, South Africa
Lorika S. Beukes: Microscopy and Microanalysis Unit, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, PieterMaritzburg 3209, South Africa

1 Introduction

The fabrication of nanoparticles through green route has attracted special attention of scientists in the growing area of nanotechnology because of the simplicity of procedure, the nonuse of hazardous chemicals or techniques, the low consumption of energy, and the increased level of biocompatibility for applications in the living systems. In addition, the large-scale production of nanoparticles can be easily achieved through this means, whereas the abundance of biomolecules that can serve as bioreductants in the green synthesis of nanoparticles in diverse living things also contributes enormously to the growing trend in green nanotechnology. In this regard, biomolecules obtained from bacteria [1–3], fungi [4–6], macro- and microalgae [7–10], arthropods [11, 12], and different parts of green plants [13–17] have been used to synthesize a wide range of nanoparticles under ambient conditions. We have documented the use of microbial enzymes [18, 19],

Cola nitida seeds, seed shell and pod [20, 21], cocoa pod husk [22], spider cobweb [23], and culture supernatant of *Bacillus safensis* [24] to synthesize AgNPs. These particles have shown tremendous biomedical properties.

Among diverse metallic nanoparticles that have been studied, AgNPs occupy prime position because of several applications alluded to them. Their optical, electronic, catalytic, antimicrobial, and electrochemical attributes have greatly influenced their relevance in diverse areas such as in food, health care, agriculture, biomedical, environmental, textile, and catalytic applications [25]. Suffice to say that the list of applications of AgNPs seems endless as new areas of relevance emerge very often. The green synthesis of AgNPs also contributes to the upsurge in the applications of AgNPs, as the improved compatibility with biological systems through the avoidance of hazardous procedures is achieved. Therefore, newer sources of biomolecules from diverse living entities are continually sought to catalyze the biological synthesis of nanoparticles for various applications in different areas of human endeavors. An important emerging area of application of biosynthesized AgNPs is in the management of blood coagulation disorders, whereby nanoparticles can be used to prevent aggregation of platelets [26] to inhibit blood coagulation. Similarly, nanoparticles can be used alone or as carriers of active drugs for the efficient and timely dissolution of blood clots, thereby preventing the untold outcome of the formation of blood clot (thrombus), which may include ischemia or stroke arising from blood coagulation and cardiovascular disorders.

Miracle fruit plant (*Synsepalum dulcificum*) is a shrub tree native to the rain forest zone of Africa and popularly known for the production of a glycoprotein sweetener (miraculin) in its fruit. The plant is found in Nigeria, Ghana, Benin, Cameroun, and Congo [27]. *S. dulcificum* is rich in a lot of phytochemicals such as miraculin responsible for the intense sweetness of its fruit, (+)-episyringaresinol, vanillin, quercetin-3-monogalactoside, and cyanidin-3-monogalactoside for diverse food/health usefulness [27]. The fruit as the source of miraculin can be used for the control of diabetes, whereas several other active principles, including pigment and phytochemicals, can be formulated into pharmaceutical products [27]. Owing to richness in biologically active phytochemicals, different parts of the plant have been used in folklore medicine. The leaves have been reportedly used to treat diabetes, malaria, hyperthermia, hemorrhoids, and enuresis, and the seeds can be used for the treatment of stomach ache, anemia, and obesity [28, 29]. Similarly, the roots of the plant have been used in the treatment of cough and tuberculosis, and the bark is used to manage prostate problems [28]. In this work, the usefulness of leaf and seed extracts of *S. dulcificum* to synthesize AgNPs as well as the evaluation of the antibacterial, antifungal, dye decolorization, anticoagulant, and thrombolytic actions of the biosynthesized nanoparticles were conducted. To the best of our knowledge, this work is the first to report the utilization of *S. dulcificum* to synthesize nanoparticles.

2 Materials and methods

2.1 Source and processing of leaf and seeds of *S. dulcificum*

Fresh leaves and berries of *S. dulcificum* were obtained from the shrub plant growing on a farm at Ipetumodu, Osun State, Southwestern Nigeria. The leaves and seeds were dried at room temperature (30°C ± 2°C) for 5 days, after which dried samples were blended into powder. The powdered samples were kept at room temperature for further use.

2.2 Extraction of biomolecules from plant parts

The powdered samples were extracted with NaOH, by dissolving 0.1 g of sample in 10 ml of 0.1 M NaOH, and heated for 1 h at 60°C. The mixtures were cooled to room temperature, followed by centrifugation at 4000 rpm for 30 min. The hydrolysates obtained were used without further purification.

2.3 Phytosynthesis and characterization

Extracts obtained from the leaf and seeds of *S. dulcificum* were used for the phytosynthesis of AgNPs using previously described procedures [20, 21] by allowing 1 ml of plant extract to react with 40 ml of 1 mM of silver nitrate (Merck, Darmstadt, Germany) under ambient condition. The timely development and stabilization of color as function of the formation of AgNPs were monitored. The colloidal AgNPs were subjected to UV-Vis spectroscopy by scanning the absorbance within 240–850 nm on spectrophotometer (Cecil, Cambridge, UK), whereas Fourier transform infrared (FTIR) spectra were obtained on IRAffinity-1S Spectrometer (Shimadzu, Milton Keynes, UK). TEM analysis was conducted using JEM-1400 (JEOL, Peabody, MA, USA) operated at 200 kV as previously described [22].

2.4 Antibacterial and antifungal assay

Both leaf and seed-mediated AgNPs were investigated for their antimicrobial activities using some established cultures in our laboratory. The antibacterial studies involved the use of drug-resistant strains of *Pseudomonas aeruginosa* and *Klebsiella granulomatis* through agar diffusion assay as stated earlier [21, 22], by dispensing 100 µl of different concentrations of particles into bored wells on Mueller-Hinton agar plates that have been seeded with overnight grown cultures of the test bacteria. Growth inhibitions were monitored by incubating the cultures at 37°C for 24 h. In the antifungal assay, the method of Khatami et al. [30] was used by incorporating 100 µg/ml of the AgNPs into potato dextrose agar (PDA). Thereafter, fungal plugs of 6-mm diameter 72-h-old cultures of *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* were used to inoculate PDA plates at the center. The incubation of the fungal cultures was done at room temperature (30°C ± 2°C) for 72 h to observe for growth. The control plates that were not exposed to AgNPs and those exposed to the extracts were also set up. The percentage growth inhibition was obtained; thus,

$$\frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100\%,$$

where D represents the measurement of fungal radial growth.

2.5 Degradation of dye

The degradation of malachite green was undertaken by mixing 20 µg/ml of AgNPs with malachite green (40 ppm) in a 1:10 ratio. Mixtures of the dye and extracts alone were set up as control samples. The mixtures were kept on rotary shaker at 100 rpm for up to 24 h. At periodic intervals, samples were taken, and absorbance readings were obtained. The degradation of malachite green was determined; thus,

$$\text{Percentage dye degradation} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100\%,$$

where A is the absorbance at 619 nm.

2.6 Blood anticoagulation and thrombolytic activities

AgNPs were investigated for their anticoagulant properties using the blood sample freely given by a healthy donor. Using AgNPs of 100 µg/ml, 0.5 ml was dispensed in clean, grease-free bottle, to which fresh human blood (5 ml) was added. A series of control experiments were set up, and these included a collection of blood into an ethylenediaminetetraacetic acid (EDTA) container (positive control) and an ordinary clean bottle (negative control). All the experiments were conducted at 30°C ± 2°C and monitored for the formation of blood clot. Microscopic investigation of the samples was undertaken by smearing blood samples on clean, grease-free slides and observed under the Olympus microscope.

The determination of thrombolytic activities of AgNPs was conducted as previously described [31] by treating preformed blood clots with 0.2 ml of 100 µg/ml AgNPs on the slide. Similarly, control samples were set up by treating blood clots with the extracts of *S. dulcificum* and $AgNO_3$ solution. All the experiments were conducted at 30°C ± 2°C, and thrombolysis (dissolution of blood clot) was monitored. Microscopic images of the contents were also obtained.

3 Results and discussion

3.1 Biosynthesized AgNPs and their characteristics

Both leaf and seed extracts of *S. dulcificum* catalyzed the formation of AgNPs (Figure 1), leading to the development of brown colloidal solution within 5 min. The color stabilized in 10 min, with more intense color recorded in leaf AgNPs. The change in color of metallic salt solutions is a manifestation of the formation of nanoparticles. The formations of different shades of color, including light yellow and yellow brown to dark brown colloidal AgNPs, are widely reported in literature [1, 18, 20, 23, 24, 32, 33]. The nature of bioreductant molecules largely influences the excitation of reduced particles, thereby leading to the development of different shades of color.

The nanoparticles absorbed maximally at wavelengths of 440 and 438.5 nm for leaf and seed AgNPs, respectively; however, higher absorbance was obtained for leaf AgNPs as a function of intense color formation (Figure 2). The broadness of the spectra may be indicative of fair incidence of polydispersed particles. The absorbance values are within the range of 431, 436, 454.5, and 457.5 nm obtained for AgNPs biosynthesized using *C. nitida* pod, cobweb, *C. nitida* seed shell, and seed extracts, respectively [20, 21, 23]. The reddish brown colloidal AgNPs produced by the leaf extract of pineapple also yielded maximum absorption

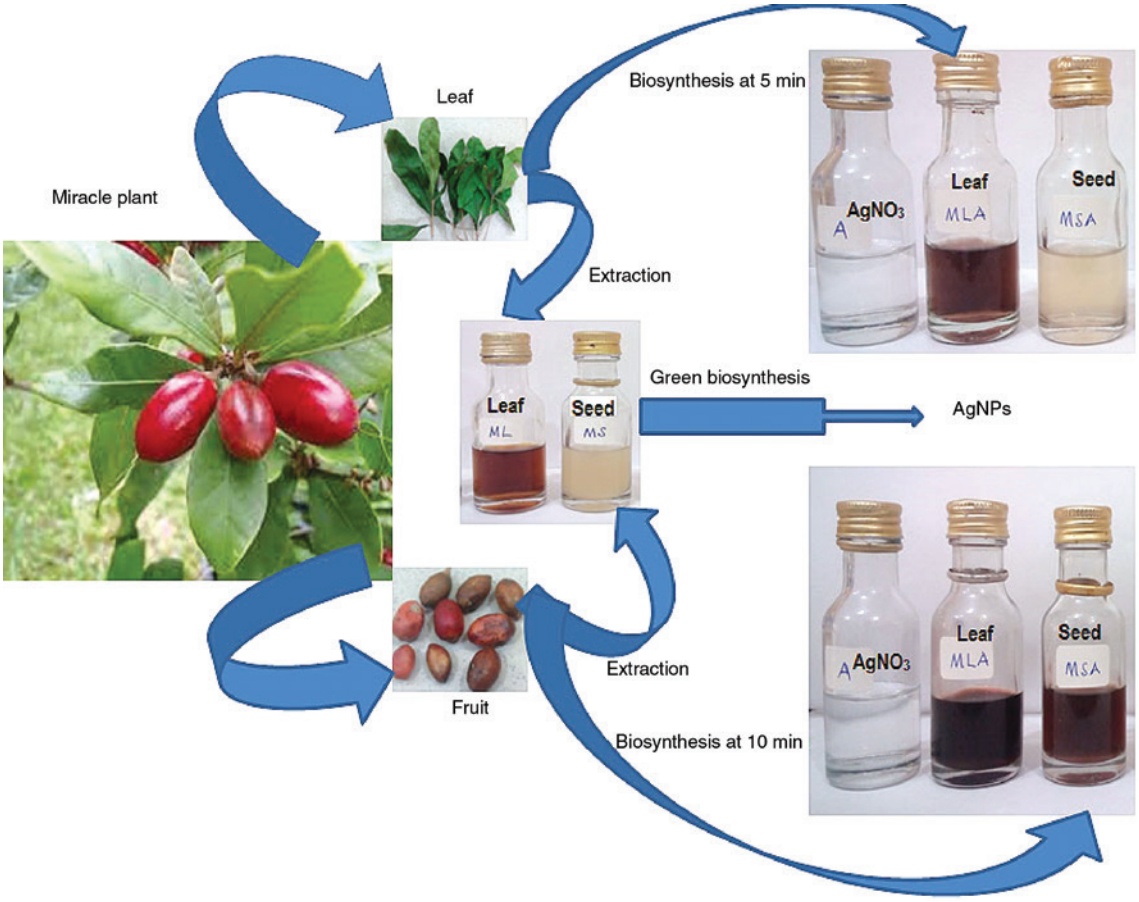

Figure 1: Schematic view of the biosynthesis of AgNPs using leaf and seed extracts of *S. dulcificum*.

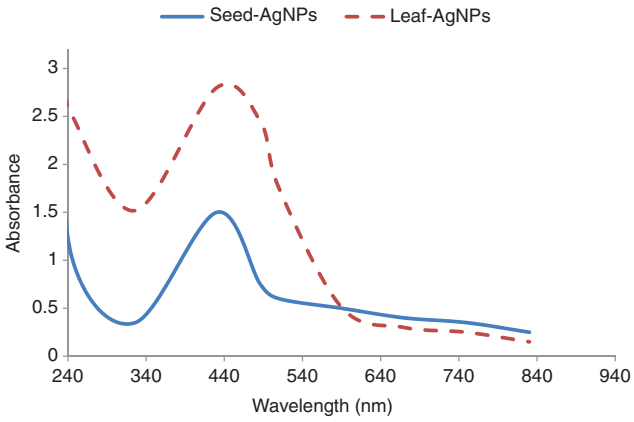

Figure 2: UV-Vis absorption of the biosynthesized AgNPs using leaf and seed extracts of *S.dulcificum*.

between 440 and 460 nm [32]. The higher absorbance readings obtained for leaf AgNPs clearly indicated the formation of more nanoparticles than in the seed AgNPs, and this may be attributed to the abundance of bioreductant molecules in the leaf of *S. dulcificum*.

The FTIR analysis revealed prominent peaks at 3408, 2357, 2089, and 1639 cm^{-1} for leaf AgNPs, whereas peaks at 3404, 2368, 2081, and 1641 cm^{-1} were obtained for seed AgNPs (Figure 3). In addition, smaller peaks at 1483, 1465, 1117, 517, 487, 437, and 417 cm^{-1} were recorded. The peaks 3404 and 3408 cm^{-1} are attributed to the O-H [34] or H-bonded bands of phenolics or alcoholic compounds. The peaks 2357 and 2368 cm^{-1} are related to vibrations of NH^{2+}/NH^{3+} in the peptide bonds of protein molecules [35] or nitrogen-rich compounds for instance nitriles (C-N) and cyanates (O-CN) [36], whereas 1639 and 1641 cm^{-1} are assigned N-H bend in primary amine [37]. Similarly, peaks occurring at 1465–1483 cm^{-1} are indicative of C-C stretch in aromatic compounds, whereas the peak 1117 cm^{-1} found only in the seed AgNPs is attributed to C-N stretch present in aliphatic amines. All these indicate that the hydroxyl group of phenolic compounds and proteins in the extracts might have formed layers on the particles as capping molecules to prevent aggregation of the AgNPs. Leaves of *S. dulcificum* have been reported to contain several bioactive materials such as β-sitosterol, stigmasterol, pheophytin-a

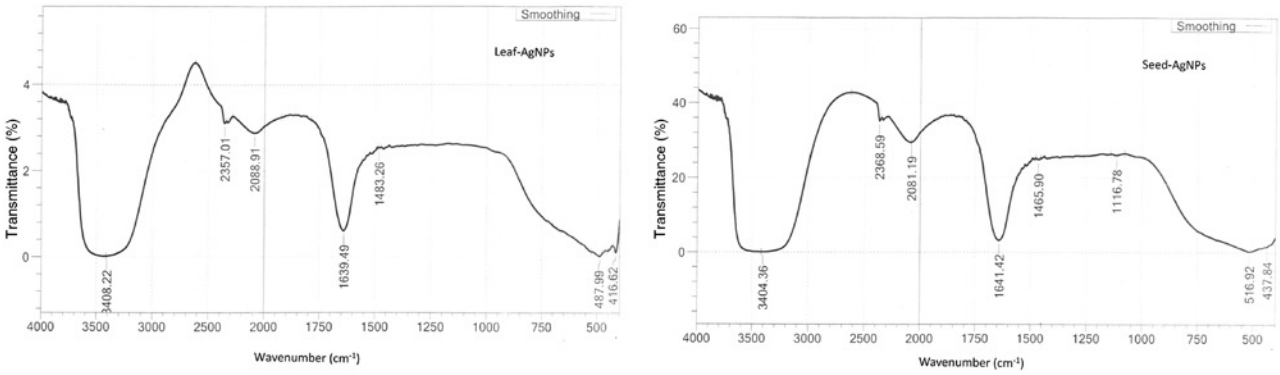

Figure 3: FTIR spectra of the biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum*.

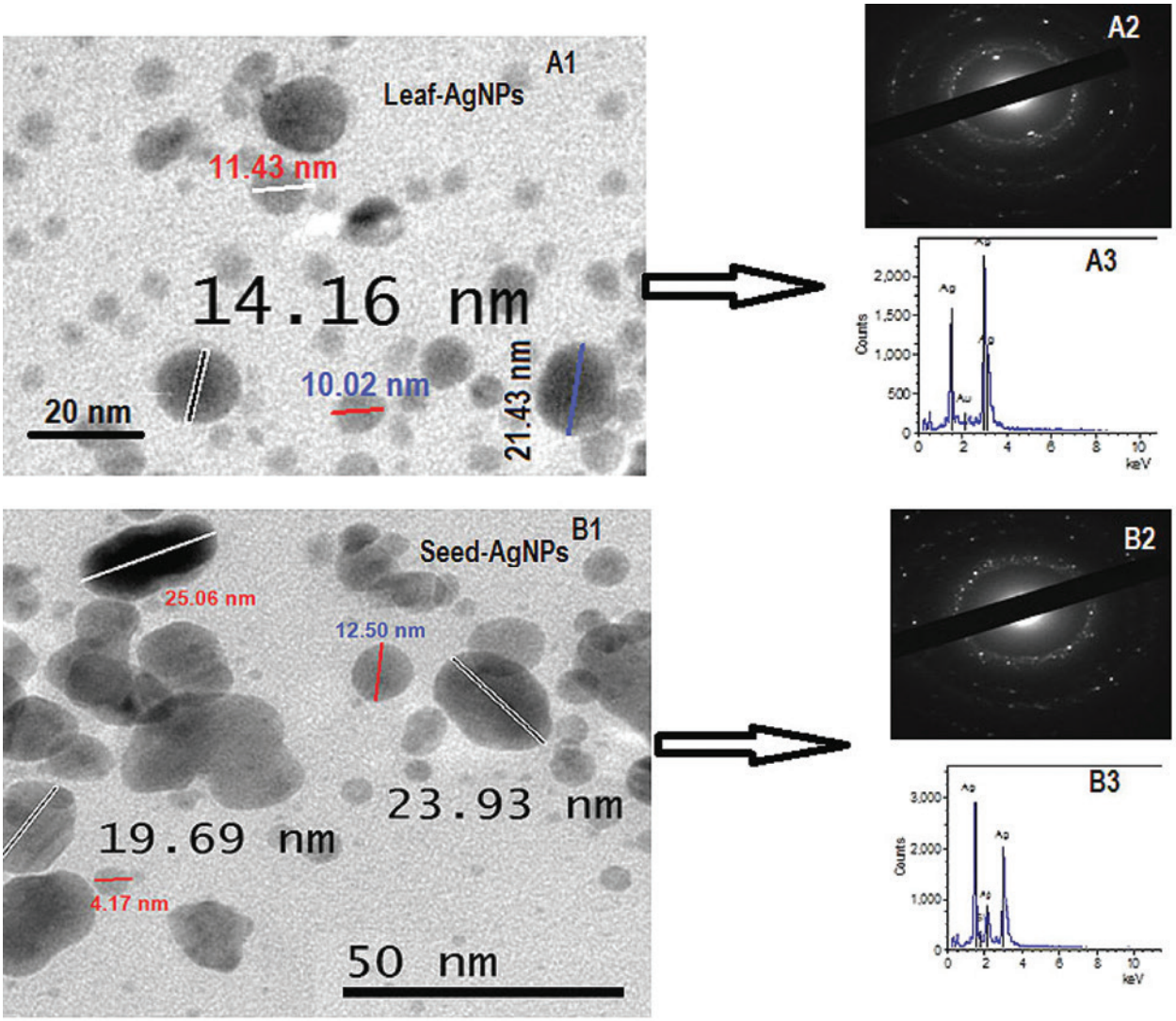

Figure 4: TEM images (A1, B1), selected electron area diffraction (A2, B2), and energy-dispersive X-ray spectroscopic analysis (EDX) patterns (A3, B3) of the biosynthesized AgNPs using leaf (A) and seed (B) extracts of *S. dulcificum*.

and b, lupeol, lupenone, α-tocopherol, and quinine [38], whereas the seed is abundantly rich in phenolics and proteins [39, 40]. It is evident from the foregoing that the leaves and seeds of *S. dulcificum* are rich in biomolecules that served as bioreductants to synthesize AgNPs.

Transmission electron micrographs (Figure 4) showed the formation fairly spherical AgNPs having size of 5–22 and 4–26 nm for leaf AgNPs and seed AgNPs, respectively. The leaf AgNPs (Figure 4A1) were well dispersed, whereas little agglomeration was noticed in seed AgNPs (Figure 4B1), which might be as a result of sedimentation at the latter stage of synthesis as similarly observed by Ahmad et al. [36]. The agglomeration brought about few incidences of nonspherical and bigger particles. The selected electron area diffraction showed that the AgNPs had crystalline nature (Figure 4A2 and B2) typical of Ag as shown by the ringlike patterns [41], whereas EDX indicated prominence of Ag (Figure 4A3 and B3).

3.2 Antimicrobial activities

The AgNPs inhibited the growth of *P. aeruginosa* and *K. granulomatis* (Figure 5) producing the zone of inhibition of 11–24 mm with MIC of 60 µg/ml. However, the plant extracts were not active against the drug-resistant isolates. The antibacterial activity of AgNPs is well established in literature [7, 18, 20, 21, 23, 24, 32, 41] as obtained in this study. It has been postulated that AgNPs can induce killing in bacteria through interactions with sulfur and phosphorus containing constituents in the cell wall, thereby disrupting respiration and growth/reproduction [42]. However, it is worth mentioning that AgNPs produced in this work acted against resistant bacteria, indicating that the particles may have biomedical importance to combat drug-resistant bacteria in the environment. The scourge of resistant bacteria is appalling as previously established in some of our investigations [43–46]. Similarly, the particles showed excellent activities against the fungal isolates (Figure 6), producing 100% suppression of the growth of *A. flavus* and *A. niger*, whereas leaf and seed AgNPs produced inhibitions of 75.60% and 73.17%, respectively, against *A. fumigatus*. There was no inhibition of growth in control experiments. These results are in agreement with those earlier established [21, 30, 33]. AgNPs of 40 µg/ml were reported to inhibit the growth of *Neofusicoccum parvum* by 83% [30]. Results of the present work showed that AgNPs can

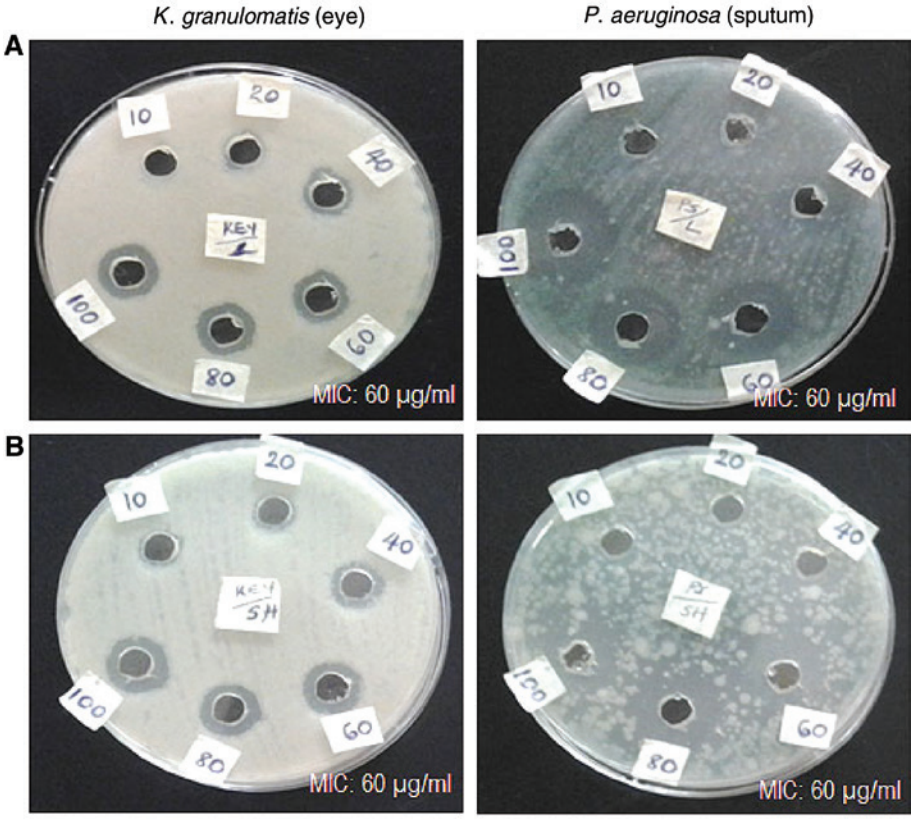

Figure 5: Antibacterial activities of the biosynthesized AgNPs using leaf (A) and seed (B) extracts of *S. dulcificum*.

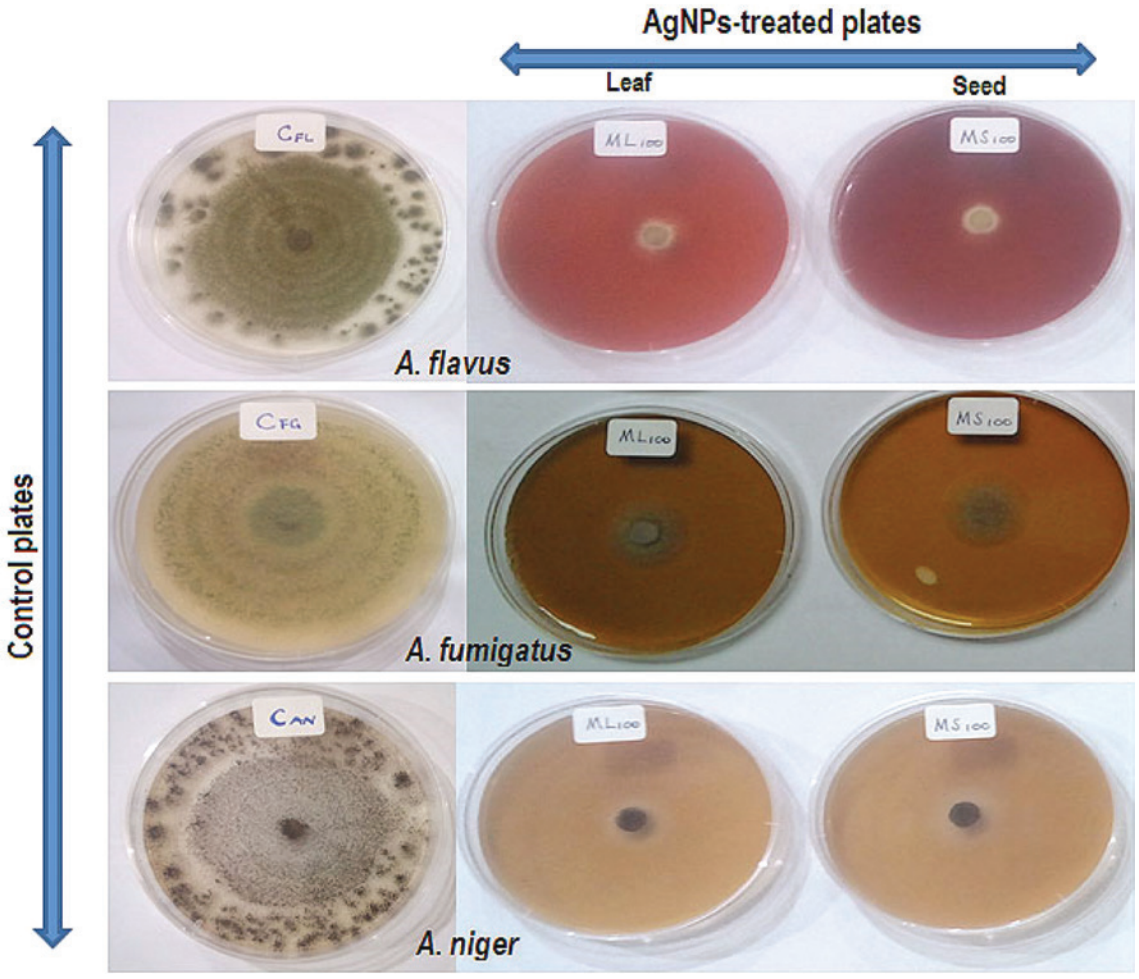

Figure 6: Antifungal activities of the biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum*.

serve as potent antifungal agents. The antifungal activities can be a result of cell wall attack and destruction of spores, which promote the outflow of intracellular constituents and, consequently, death.

3.3 Degradation of malachite green by the biosynthesized AgNPs

The AgNPs degraded malachite green at working concentration of 20 µg/ml, producing approximately 80% reduction within 24 h (Figure 7). The degradation was steady and concentration dependent in both leaf and seed AgNPs, whereas exposure to extracts alone did not lead to degradation of malachite green (data not shown). The use of nanoparticles as obtained in this study for the degradation of dyes is reported in literature [47–49], with some advantages over conventional techniques of absorption, adsorption, coagulation, flocculation, ultrafiltration, reverse osmosis, and membrane technologies that only involve concentration or transferring of organic compounds from one form to another [50]. Using $NaBH_4$, 100% catalytic reduction was achieved with 29.4 µg/ml of *Sterculia acuminata* fruit extract-mediated gold nanoparticles in 12 min [48], whereas Kumari and Philip [49] reported 83% and 95% degradation of methylene blue within 12 min, using *Punica granatum* fruit extract-mediated AgNPs and AuNPs, respectively, under the influence of $NaBH_4$. However, in the present study, the catalytic degradation of malachite green was achieved without the use of $NaBH_4$ and exposure to light. Within 3 h, the degradation of malachite green at 67.7% and 72.5% was achieved by the leaf and seed-mediated AgNPs, respectively. The degradation showed little improvements to 80% and 78.6% in the next 21 h, which may be attributed to the early attainment of equilibrium. The underlying mechanism of dye degradation of dyes by nanoparticles had been captured

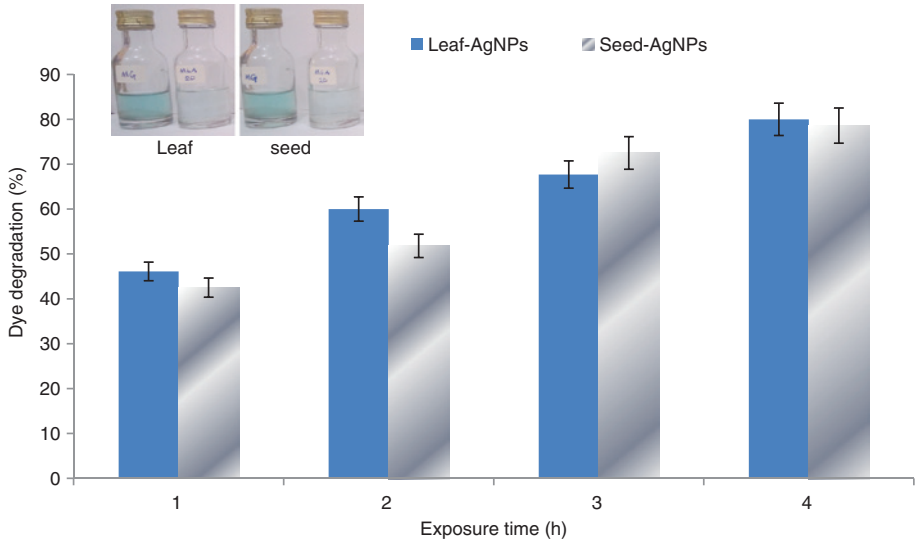

Figure 7: Degradation of malachite green by biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum* (inset, degradation at 2 h).

in an electron relay effect [51], whereby nanoparticles mediate in transferring electron between biomolecules borne on nanoparticles and the dye for the catalytic degradation of dye. Results obtained in this work have further shown the practicability of the applications of biosynthesized AgNPs for the catalytic degradation of malachite green.

3.4 Anticoagulant and thrombolytic activities of biosynthesized AgNPs

Both leaf and seed AgNPs displayed excellent blood anticoagulant activities (Figure 8), which prevented the blood samples from clotting. The microscopic examination revealed the presence of well-dispersed red blood cells,

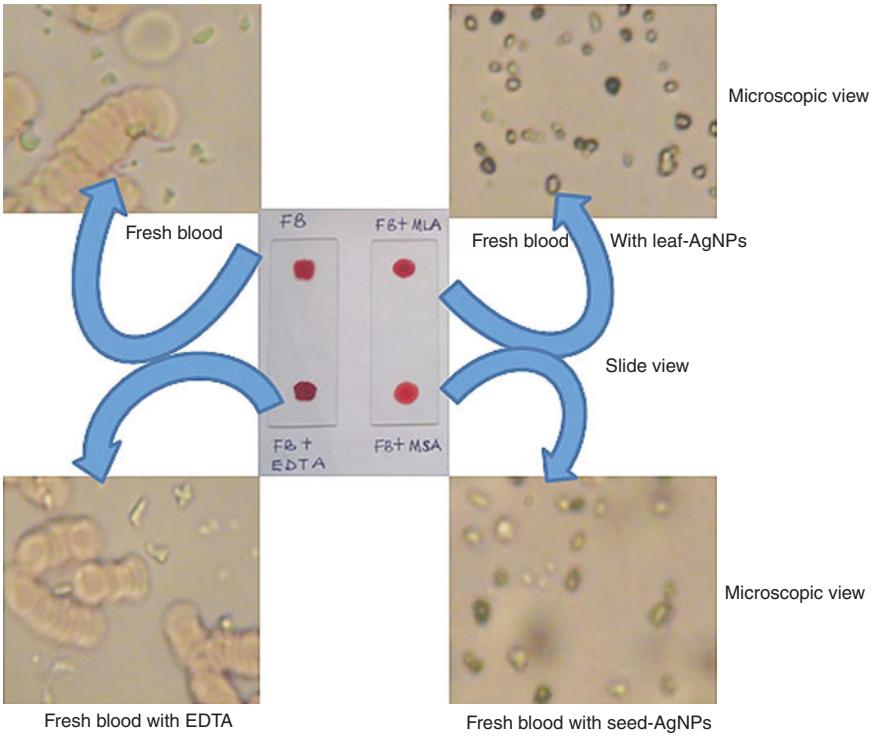

Figure 8: Blood anticoagulant activities of the biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum*.

which are comparable with those obtained using the conventional EDTA blood anticoagulant. The observed structural change of red blood cells in blood treated with AgNPs as against the biconcave forms in EDTA-treated blood may be attributed to the nature of the biosynthesized AgNPs, particularly the concentration used and the pH level of the colloidal solution. We are currently focusing on the optimization of these parameters to produce combinations that can prevent blood coagulation and maintain the morphology of blood cells and biochemical attributes of the blood. The essence of blood coagulation system in the maintenance of steady blood flow, the prevention of bleeding, and the prevention of the spread of infectious agents [52] by assisting the innate immune system is well established. However, the system can also portend some troubles as the blood clots arising from infections are capable of destroying tissues, which may ultimately affects organs [53]. This can be seen in cardiovascular diseases, immunological disorders, wounds, and onset of tumor [54, 55]. Also, developed cancer cells can initiate the formation of blood clot by stimulating proinflammatory cytokines, the production of procoagulants, and the interaction with blood platelets [56, 57]. These problems have shown the necessity to control blood coagulation disorders, which can be achieved through nanotechnology. In a previous study, Shrivastava et al. [26] used AgNPs to prevent the aggregation of platelets, thereby forestalling the formation of blood clot (thrombus). The study also established that AgNPs were not toxic to the blood platelets. Therefore, the nontoxic nature of AgNPs to platelets and its established actions against microbes can represent a paradigm shift in preventing blood coagulation. Most recently, Kim et al. [58], reported improvement in the anticoagulant activities of heparin coupled with

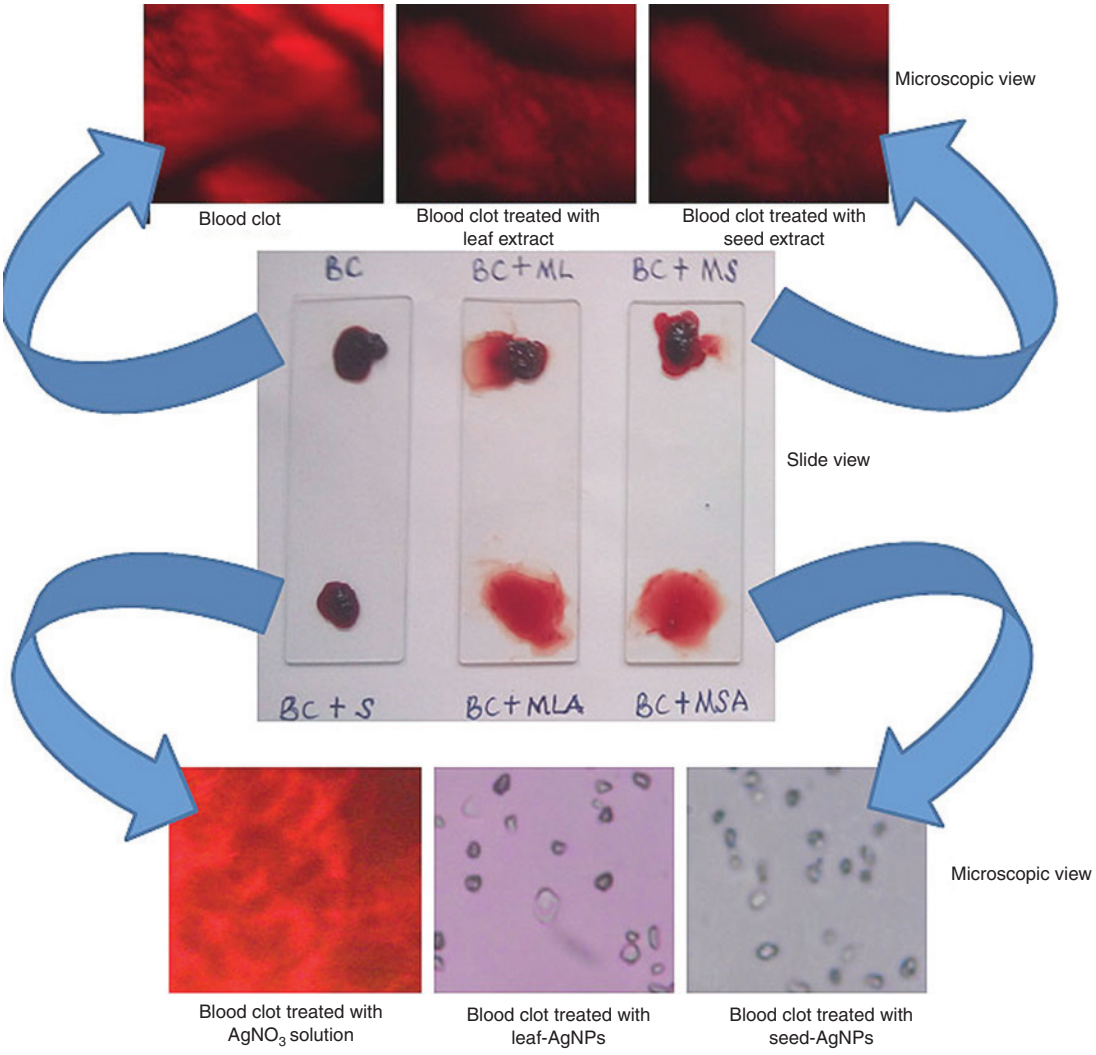

Figure 9: Thrombolytic activities of the biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum*.

gold nanoparticles biosynthesized using the earthworm extract. The heparin AuNPs produced a 118.9% improvement on the clotting time of heparin alone. The potential of AgNPs as a potential anticoagulant is further established in the present study.

The AgNPs dissolved preformed blood clots within 2 min, providing clear blood fluid on the slides (Figure 9). When viewed under the microscope, the dispersion of red blood cells was seen in AgNP-treated blood clots as opposed to the negative controls, where the $AgNO_3$ solution and leaf and seed extracts of *S. dulcificum* failed to dissolve the blood clots. The results showed clearly the thrombolytic activities of both leaf and seed AgNPs, which are similar to the report of Harish et al. [31]. Blood clotting is nature's antidote to excessive bleeding, but its timely dissolution is needed to prevent thrombosis and maintain homeostasis [59], and this can be aptly achieved by optimizing treatment through the application of nanomaterials. The long-established anticoagulant management strategy, for instance, the use of streptokinase, is plagued with problems of short half-life, neutralization of the foreign agents through the activities of antibodies, and danger of extreme bleeding. These problems can be solved through the applications of nanotechnology, whereby nontoxic and biocompatible nanoparticles can be used as either stand-alone thrombolytic agents or carriers of active drugs. The possible mechanisms of thrombolysis by AgNPs is presented in Figure 10, whereby the nanoparticles can act directly on fibrin (mechanism 2) to break it down as evidenced from the plate assay of Harish et al. [31]. By contrast, AgNPs can act on plasminogen, causing its activation to release plasmin that then breaks the blood clot (mechanism 1). In addition, the nanoparticles can inhibit the activities of inhibitors that may prevent the activation of plasminogen and plasmin. It is envisaged that the two mechanisms could occur together, thereby leading to the pronounced thrombolytic activities as obtained in this study. Although there is limited report on the exploitation of AgNPs as a thrombolytic material, the present investigation showed that it can serve as a thrombolytic nanoagent in nanomedicine. Most recently, we have shown that Ag, Au and Ag-AuNPs possessed anticoagulant and thrombolytic activities [41, 60–62], with potential for biomedical applications.

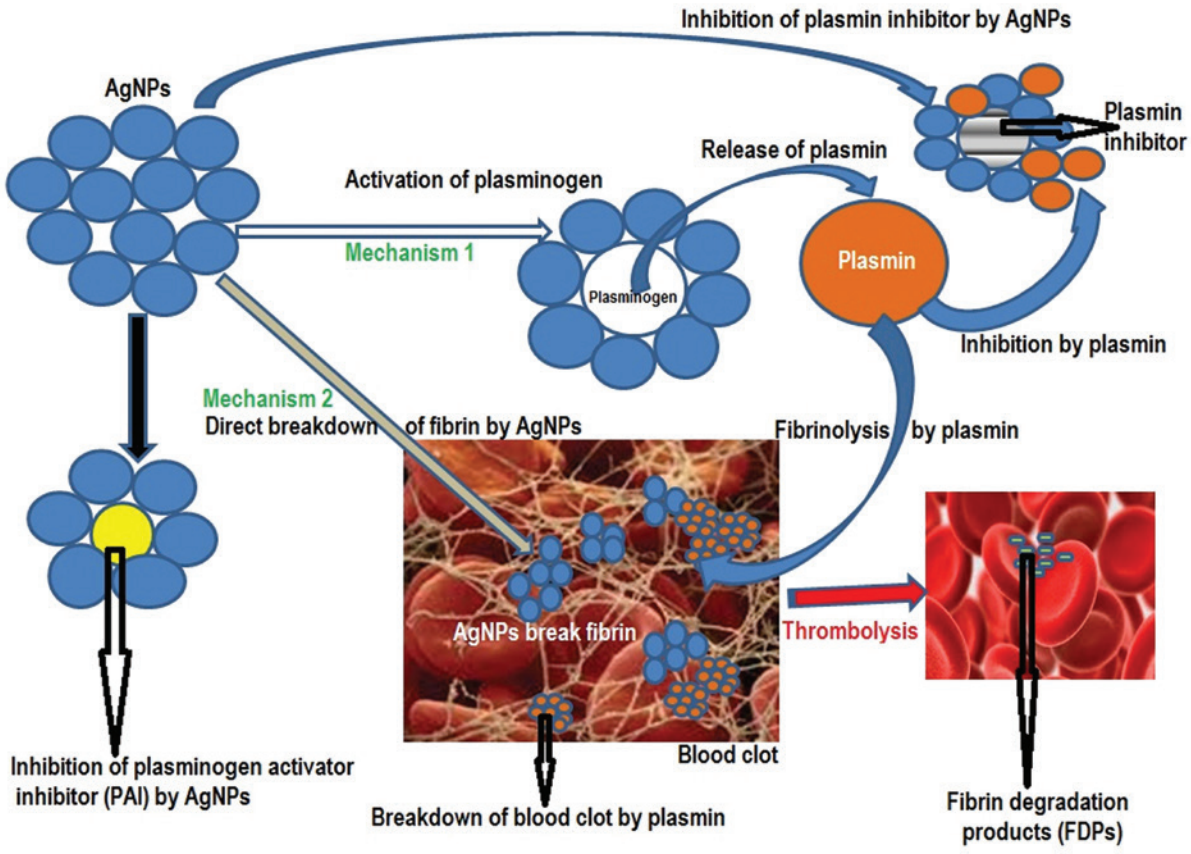

Figure 10: The possible mechanisms of thrombolytic activities of the biosynthesized AgNPs.

4 Conclusion

This work has demonstrated the phytosynthesis of AgNPs using leaf and seed extracts of *S. dulcificum*, leading to the production of fairly spherical particles of 4–26 nm in size. The particles showed maximum absorption at wavelengths of 438.5 and 440 nm for the leaf and seed AgNPs, respectively. The rich phytochemicals in the extracts, particularly phenolics and proteins, took part in the synthesis of particles as evidenced from the FTIR spectra. The two AgNPs showed comparative activities at inhibiting resistant bacterial strains and fungal strains of *A. niger, A. flavus*, and *A. fumigatus*. Similarly, the particles achieved approximately 80% degradation of malachite green under ambient conditions, whereas potent blood anticoagulant and thrombolytic activities were displayed by the AgNPs. It is therefore evident that AgNPs could be applied as antimicrobial agents, as nanocatalysts to degrade dyes in wastewater/effluents, and in nanomedicine for the management of blood coagulation disorders. As far as we know, the study is the first to demonstrate the synthesis of AgNPs using the metabolites of *S. dulcificum* with potent antimicrobial, catalytic, anticoagulant, and thrombolytic activities.

Acknowledgments: A. Lateef is deeply grateful to the management of LAUTECH, Ogbomoso, Nigeria, for providing the equipment used in this study.

References

[1] Das VL, Thomas R, Varghese RT, Soniya EV, Mathew J, Radhakrishnan EK. Extracellular synthesis of silver nanoparticles by the *Bacillus* strain CS 11 isolated from industrialized area. *3 Biotech*. 2014, 4, 121–126.

[2] Dehnad A, Hamedi J, Derakhshan-Khadivi F, Abusov R. Green synthesis of gold nanoparticles by a metal resistant *Arthrobacter nitroguajacolicus* isolated from gold mine. *IEEE Trans. NanoBiosci.* 2015, 14, 393–396.

[3] Jang GG, Jacobs CB, Gresback RG, Ivanov IN, Meyer III HM, Kidder M, Joshi PC, Jellison GE, Phelps TJ, Graham DE, Moon JW. Size tunable elemental copper nanoparticles: extracellular synthesis by thermoanaerobic bacteria and capping molecules. *J. Mater. Chem. C*. 2015, 3, 644–650.

[4] El-Batal AI, ElKenawya NM, Yassin AS, Amin MA. Laccase production by *Pleurotus ostreatus* and its application in synthesis of gold nanoparticles. *Biotechnol. Reports* 2015, 5, 31–39.

[5] Mishra A, Kumari M, Pandey S, Chaudhry V, Gupta KC, Nautiyal CS. Biocatalytic and antimicrobial activities of gold nanoparticles synthesized by *Trichoderma* sp. *Bioresour. Technol.* 2014, 166, 235–242.

[6] Waghmare SR, Mulla MN, Marathe SR, Sonawane KD. Ecofriendly production of silver nanoparticles using *Candida utilis* and its mechanistic action against pathogenic microorganisms. *3 Biotech* 2015, 5, 33–38.

[7] Kathiraven T, Sundaramanickam A, Shanmugam N, Balasubramanian T. Green synthesis of silver nanoparticles using marine algae *Caulerpa racemosa* and their antibacterial activity against some human pathogens. *Appl. Nanosci.* 2015, 5, 499–504.

[8] Patel V, Berthold D, Puranik P, Gantar M. Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. *Biotechnol. Reports* 2015, 5, 112–119.

[9] Sinha SN, Paul D, Halder N, Sengupta D, Patra SK. Green synthesis of silver nanoparticles using fresh water green alga *Pithophora oedogonia* (Mont.) Wittrock and evaluation of their antibacterial activity. *Appl. Nanosci.* 2015, 5, 703–709.

[10] Suganya KU, Govindaraju K, Kumar VG, Dhas TS, Karthick V, Singaravelu G, Elanchezhiyan M. Blue green alga mediated synthesis of gold nanoparticles and its antibacterial efficacy against Gram positive organisms. *Mater. Sci. Eng. C* 2015, 47, 351–356.

[11] Xu S, Yong L, Wu P. One-pot, green, rapid synthesis of flower-like gold nanoparticles/reduced graphene oxide composite with regenerated silk fibroin as efficient oxygen reduction electrocatalysts. *ACS Appl. Mater. Interf.* 2013, 5, 654–662.

[12] Aramwit P, Bang N, Ratanavaraporn J, Ekgasit S. Green synthesis of silk sericin-capped silver nanoparticles and their potent anti-bacterial activity. *Nanoscale Res. Lett.* 2014, 9, 1–7.

[13] Kumar DA, Palanichamy V, Roopan SM. Green synthesis of silver nanoparticles using *Alternanthera dentata* leaf extract at room temperature and their antimicrobial activity. *Spectrochim. Acta Part A. Mol. Biomol. Spectr.* 2014, 127, 168–171.

[14] Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S. Agricultural waste *Annona squamosa* peel extract: biosynthesis of silver nanoparticles. *Spectrochim. Acta Part A. Mol Biomol. Spectr*. 2012, 90, 173–176.

[15] Naraginti S, Kumari PL, Das RK, Sivakumar A, Patil SH, Andhalkar VV. Amelioration of excision wounds by topical application of green synthesized, formulated silver and gold nanoparticles in albino Wistar rats. *Mater. Sci. Eng. C* 2016, 62, 293–300.

[16] Nayak D, Ashe S, Rauta PR, Kumari M, Nayak B. Bark extract mediated green synthesis of silver nanoparticles: evaluation of antimicrobial activity and antiproliferative response against osteosarcoma. *Mater. Sci. Eng. C* 2016, 58, 44–52.

[17] Roopan SM, Madhumitha G, Rahuman AA, Kamaraj C, Bharathi A, Surendra TV. Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. *Ind. Crops Prod*. 2013, 43, 631–635.

[18] Lateef A, Adelere IA, Gueguim-Kana EB, Asafa TB, Beukes LS. Green synthesis of silver nanoparticles using keratinase obtained from a strain of *Bacillus safensis* LAU 13. *Int. Nano Lett.* 2015, 5, 29–35.

[19] Lateef A, Adeeyo AO. Green synthesis and antibacterial activities of silver nanoparticles using extracellular laccase of *Lentinus edodes*. *Not. Sci. Biol.* 2015, 7, 405–411.

[20] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Ajetomobi FE, Gueguim-Kana EB, Beukes LS. *Cola nitida*-mediated biogenic synthesis of silver nanoparticles using seed and seed shell extracts and evaluation of antibacterial activities. *BioNanoSci.* 2015, 5, 196–205.

[21] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Azeez L, Ajibade SE, Ojo SA, Gueguim-Kana EB, Beukes LS. Biogenic synthesis of silver nanoparticles using pod extract of *Cola nitida*: antibacterial, antioxidant activities and application as additive paint. *J. Taibah Univ. Sci.* 2016, 10, 551–562.

[22] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Azeez L, Ojo SA, Gueguim-Kana EB, Beukes LS. Cocoa pod extract-mediated biosynthesis of silver nanoparticles: its antimicrobial, antioxidant and larvicidal activities. *J. Nanostruct. Chem.* 2016, 6, 159–169.

[23] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Gueguim-Kana EB, Beukes LS. Cobweb as novel biomaterial for the green and eco-friendly synthesis of silver nanoparticles. *Appl. Nanosci.* 2016, 6, 863–874.

[24] Lateef A, Ojo SA, Akinwale AS, Azeez L, Gueguim-Kana EB, Beukes LS. Biogenic synthesis of silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13: antimicrobial, free radical scavenging and larvicidal activities. *Biologia* 2015, 70, 1295–1306.

[25] Keat CL, Aziz A, Eid AM, Elmarzugi NA. Biosynthesis of nanoparticles and silver nanoparticles. *Bioresour. Bioprocess.* 2015, 2, 47.

[26] Shrivastava S, Bera T, Singh SK, Singh G, Ramachandrarao P, Dash D. Characterization of antiplatelet properties of silver nanoparticles. *ACS Nano* 2009, 3, 1357–1364.

[27] Achigan-Dako EG, Tchokponhoué DA, N'Danikou S, Gebauer J, Vodouhè RS. Current knowledge and breeding perspectives for the miracle plant *Synsepalum dulcificum* (Schum. et Thonn.) Daniell. *Genet. Resour. Crop Evol.* 2015, 62, 465–476.

[28] Oumorou M, Dah-Dovonon J, Aboh BA, Hounsoukaka M, Sinsin B. Contribution á la conservation de *Synsepalum dulcificum*: régénération et importance socio-économique dans le département de l'ouémé (Bénin). *Ann. Sci. Agron.* 2010, 14, 101–120.

[29] Soladoye MO, Adetayo MO, Chukwuma EC, Adetunji AN. Ethnobotanical survey of plants used in the treatment of haemorrhoids in South-Western Nigeria. *Ann. Biol. Res.* 2010, 1, 1–15.

[30] Khatami M, Pourseyedi S, Khatami M, Hamidi H, Zaeifi M, Soltani L. Synthesis of silver nanoparticles using seed exudates of *Sinapis arvensis* as a novel bioresourse, and evaluation of their antifungal activity. *Bioresour. Bioprocess.* 2015, 2, 19.

[31] Harish BS, Uppuluri KB, Anbazhagan V. Synthesis of fibrinolytic active nanoparticles using wheat bran xylan as a reducing and stabilizing agent. *Carbohydr. Polymer* 2015, 132, 104–110.

[32] Emeka EE, Ojiefoh OC, Aleruchi C, Hassan LA, Christiana OM, Rebecca M, Dare EO, Temitope AE. Evaluation of antibacterial activities of silver nanoparticles green-synthesized using pineapple leaf (*Ananas comosus*). *Micron* 2014, 57, 1–5.

[33] Netala VR, Kotakadi VS, Domdi L, Gaddam SA, Bobbu P, Venkata SK, Ghosh SB, Tartte V. Biogenic silver nanoparticles: efficient and effective antifungal agents. *Appl. Nanosci.* 2016, 6, 475–484.

[34] Castro L, Blázquez ML, González F, Muñoz JA, Ballester A. Extracellular biosynthesis of gold nanoparticles using sugar beet pulp. *Chem. Eng. J.* 2010, 164, 92–97.

[35] Rao A, Mahajan K, Bankar A, Srikanth R, Kumar AR, Gosavi S, Zinjarde S. Facile synthesis of size-tunable gold nanoparticles by pomegranate (*Punica granatum*) leaf extract: applications in arsenate sensing. *Mater. Res. Bull.* 2013, 48, 1166–1173.

[36] Ahmad T, Wani IA, Manzoor N, Ahmed J, Asiri AM. Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. *Colloids Surf. B. Biointerf.* 2013, 107, 227–234.

[37] Yugandhar P, Haribabu R, Savithramma N. Synthesis, characterization and antimicrobial properties of green-synthesised silver nanoparticles from stem bark extract of *Syzygium alternifolium* (Wt.) Walp. *3 Biotech.* 2015, 5, 1031–1039.

[38] Chen CY, Wang YD, Wang HM. Chemical constituents from the leaves of *Synsepalum dulcificum*. *Chem. Nat. Compd.* 2010, 46, 495–495.

[39] Du L, Shen Y, Zhang X, Prinyawiwatkul W, Xu Z. Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts. *Food Chem.* 2014, 153, 279–284.

[40] Jeremiah OJ, Ilesanmi OR, Ige MM. Proximate and mineral composition of *Synsepalum dulcificum* seed. *Scientific Res. J.* 2015, 3, 2201–2797.

[41] Lateef A, Akande MA, Ojo SA, Folarin BI, Gueguim-Kana EB, Beukes LS. Paper wasp nest-mediated biosynthesis of silver nanoparticles for antimicrobial, catalytic, anti-coagulant and thrombolytic applications. *3Biotech.* 2016, 6, 140.

[42] Mahendra R, Alka Y, Aniket G. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* 2009, 27, 76–83.

[43] Lateef A. The microbiology of a pharmaceutical effluent and its public health implications. *World J. Microbiol. Biotechnol.* 2004, 20, 167–171.

[44] Lateef A, Yekeen TA, Ufuoma PE. Bacteriology and genotoxicity of some pharmaceutical wastewaters in Nigeria. *Int. J. Environ. Health* 2007, 1, 551–562.

[45] Lateef A, Davies TE, Adelekan A, Adelere IA, Adedeji AA, Fadahunsi AH. Akara Ogbomoso: microbiological examination and identification of hazards and critical control points. *Food Sci. Technol. Int.* 2010, 16, 389–400.

[46] Lateef A, Ojo MO. Public health issues in the processing of cassava (*Manihot esculenta*) for the production of '*lafun*' and the application of hazard analysis control measures. *Qual. Assur. Safety Crops Fds.* 2016, 8, 165–177.

[47] Han TH, Khan MM, Kalathil S, Lee J, Cho MH. Simultaneous enhancement of methylene blue degradation and power generation in a microbial fuel cell by gold nanoparticles. *Ind. Eng. Chem. Res.* 2013, 52, 8174–8181.

[48] Bogireddy NKR, Anand KKH, Mandal BK. Gold nanoparticles-synthesis by *Sterculia acuminata* extract and its catalytic efficiency in alleviating different organic dyes. *J. Mol. Liquids* 2015, 211, 868–875.

[49] Kumari MM, Philip D. Degradation of environment pollutant dyes using phytosynthesized metal nanocatalysts. *Spectrochim. Acta Part A. Mol. Biomol. Spectr.* 2015, 135, 632–638.

[50] Soltani N, Saion E, Hussein MZ, Erfani M, Abedini A, Bahmanrokh G, Navasery M, Vaziri P. Visible light-induced degradation of methylene blue in the presence of photocatalytic ZnS and CdS nanoparticles. *Int. J. Mol. Sci.* 2012, 13, 12242–12258.

[51] Gupta N, Singh HP, Sharma RK. Metal nanoparticles with high catalytic activity in degradation of methyl orange: an electron relay effect. *J. Mol. Catal. A. Chem.* 2011, 335, 248–252.
[52] Esmon CT, Xu J, Lupu F. Innate immunity and coagulation. *J. Thromb. Haemost.* 2011, 9, 182–188.
[53] Levi M, Schultz M, van der Poll T. Disseminated intravascular coagulation in infectious disease. *Sem. Thromb. Hemost.* 2010, 36, 367–377.
[54] Prandoni P, Falanga A, Piccioli A. Cancer, thrombosis and heparin-induced thrombocytopenia. *Thromb. Res.* 2007, 120, S137–S140.
[55] Davalos D, Akassoglou K. Fibrinogen as a key regulator of inflammation in disease. *Sem. Immunopathol.* 2012, 34, 43–62.
[56] Jurasz P, Alonso-Escolano D, Radomski M.W. Platelet-cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. *Brit. J. Pharmacol.* 2004, 143, 819–826.
[57] ten Cate H, Falanga A. Overview of the postulated mechanisms linking cancer and thrombosis. *Pathophysiol. Haemost. Thromb.* 2007, 36, 122–130.
[58] Kim HK, Choi MJ, Cha SH, Koo YK, Jun SH, Cho S, Park Y. Earthworm extracts utilized in the green synthesis of gold nanoparticles capable of reinforcing the anticoagulant activities of heparin. *Nanoscale Res. Lett.* 2013, 8, 1–7.
[59] Ilinskaya AN, Dobrovolskaia MA. Nanoparticles and the blood coagulation system. Part I: benefits of nanotechnology. *Nanomedicine* 2013, 8, 773–784.
[60] Ojo SA, Lateef A, Azeez MA, Oladejo SM, Akinwale AS, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Gueguim-Kana EB, Beukes LS. Biomedical and catalytic applications of gold and silver-gold alloy nanoparticles biosynthesized using cell-free extract of *Bacillus safensis* LAU 13: antifungal, dye degradation, anti-coagulant and thrombolytic activities. *IEEE Trans. NanoBiosci.* 2016, http://dx.doi.org/10.1109/TNB.2016.2559161.
[61] Lateef A, Ojo SA, Folarin BI, Gueguim-Kana EB, Beukes LS. Kolanut (*Cola nitida*) mediated synthesis of silver-gold alloy nanoparticles: antifungal, catalytic, larvicidal and thrombolytic applications. *J. Clust. Sci.* 2016, 27, 1561–1577.
[62] Azeez MA, Lateef A, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Gueguim-Kana EB, Beukes LS. Biomedical applications of cocoa bean extract-mediated silver nanoparticles as antimicrobial, larvicidal and anticoagulant agents. *J. Clust. Sci.* 2016, http://dx.doi.org/10.1007/s10876-016-1055-2.

Bionotes

Agbaje Lateef
Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria; and Nanotechnology Research Group (NANO⁺), Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria, Tel.: +234 8037400520, **alateef@lautech.edu.ng; agbaje72@yahoo.com**

Agbaje Lateef obtained BTech in pure and applied biology, MTech in biotechnology, and PhD in microbiology in 1997, 2001, and 2005, respectively. He has 18 years of teaching experience in the university with vast interests in microbiology and biotechnology, especially fermentation processes and enzyme technology. He has approximately 70 publications to his credits. He is currently involved in the green synthesis of nanoparticles, and he is the head of the Nanotechnology Research Group (NANO⁺) in LAUTECH, Ogbomoso, Nigeria (www.nanotech.lautech.edu.ng). His articles have enjoyed 694 citations, and he has an h-index of 14. https://scholar.google.com/citations?user=C388_KsAAAAJ&hl=en.

Monsurat A. Akande
Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

Monsurat A. Akande graduated at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, where she obtained BTech in microbiology and MSc in medical microbiology and parasitology in 2008 and 2015, respectively. She worked briefly in the Laboratory of Prof. A. Lateef, focusing on the green synthesis of silver nanoparticles. She has two published articles to her credit.

Musibau A. Azeez
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria; and Nanotechnology Research Group (NANO$^+$), Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

Musibau A. Azeez obtained BTech in pure and applied biology at the Ladoke Akintola University of Technology, Ogbomoso, in 1997. He thereafter obtained MSc and PhD in Plant Genetics and Breeding at the University of Ilorin, Ilorin, Nigeria, in 2001 and 2010, respectively. He was a DBT-TWAS postdoctoral fellow in nanobiotechnology at the Department of Chemistry, University of Pune, Pune, India, in 2013–2014. He is an applied geneticist and plant breeder with vast experience in germplasm development, conservation, and utilization. He also conducts research in population genetics, medicinal plants, and nanobiotechnology. He has more than forty published article to his credit. He is a member of Nanotechnology Research Group (NANO$^+$). https://scholar.google.com/citations?user=WSYv30AAAAAJ&hl=en.

Sunday A. Ojo
Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

Sunday A. Ojo obtained BTech in microbiology at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, in First Class Division in 2010. He is currently on MTech program under the supervision of Prof. A. Lateef, with research work focusing on nanobiotechnology. He has nine publications to his credits. https://scholar.google.com/citations?user=prGuXmcAAAAJ&hl=en.

Bolaji I. Folarin
Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

Bolaji I. Folarin obtained a BTech degree in Microbiology at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, in First Class Division in 2016, where he worked under the supervision of Prof. A. Lateef on the green synthesis of silver, gold, and silver-gold alloy nanoparticles. He currently has two publications to his credit.

Evariste B. Gueguim-Kana
Department of Microbiology, University of KwaZulu-Natal, Private Bag X01, Scottsville, PieterMaritzburg 3209, South Africa

Evariste B. Gueguim-Kana obtained BTech in pure and applied biology, MTech in biotechnology, and PhD in microbiology in 1997, 2001, and 2005, respectively, at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria. He was a faculty member in Lautech., Ogbomoso from 1998 to 2008, after which he joined the services of University of KwaZulu-Natal, South Africa, in the Department of Microbiology. He is an expert in bioprocess design, modeling, and optimization, where he has contributed immensely in the novel design of bioreactors and deployment of bioprocess control software and applications. He is currently involved in renewable energy research and green nanobiotechnology. Dr. Gueguim-Kana has more than fifty articles to his credit. https://scholar.google.com/citations?user=lBbQ8FYAAAAJ&hl=en.

Lorika S. Beukes
Microscopy and Microanalysis Unit, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, PieterMaritzburg 3209, South Africa

Lorika S. Beukes obtained an honours degree in microbiology and an MSc in applied and environmental microbiology. She is currently on a PhD program in applied and environmental microbiology at the University of KwaZulu-Natal, South Africa. She has an expertise in electron, confocal, and fluorescence microscopy. She works at the microscopy and microanalysis unit of the University of KwaZulu-Natal. Beukes has 12 publications to her credit. https://www.researchgate.net/profile/Lorika_Beukes2.