Green synthesized novel silver nanoparticles and their application as anticoagulant and thrombolytic agents: A perspective

M A Azeez¹,6*, F A Durodola¹, A Lateef²,6, T A Yekeen¹,6, A O Adubi¹, I C.Oladipo³,6, E A Adebayo⁴,6 J A Badmus⁵,6 and A O Abawulem¹

¹Laboratory of Ecotoxicology, Genetics and Nanobiotechnology, ²Laboratory of Industrial Microbiology and Nanobiotechnology, Department of Pure and Applied Biology, ³Department of Science Laboratory Technology, ⁴Department of Pure and Applied Biology, ⁵Department of Biochemistry, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

⁶Nanotechnology Research Group (NAANO⁺)

*Corresponding author: maazeez@lautech.edu.ng

Abstract

Effective management of haemostatic disorders in patients is pertinent in order to prevent complications attributable to incidences of thrombosis in atherosclerotic arteries to the heart, brain, limbs, stagnant blood in veins and heart chambers. The use of existing chemical agents are not without a number of challenges, varying from potential for unknown long-term adverse effect through lack of antidote for most agents, balance between cost and efficacy among others. However, advent of biogenic silver nanoparticles with anticoagulant and thrombolytic potentials has opened up a window of opportunity to address most of the shortcomings of the chemical synthesized anticoagulants. This review examined green synthesized novel silver nanoparticles, their characterization and possible application as anticoagulant and thrombolytic agents in the evolving area of nanomedicine.

Keywords: Silver nanoparticles, anticoagulant, thrombolysis, antiplatelet, green synthesis

1. Introduction

Nanotechnology is an emerging field of synthetic chemistry that is making tremendous impact in all aspects of human life. It is the technology of the future and has revolutionized all fields, ranging from medicines to agriculture, through environment to electronics by providing abilities that would never have been previously dreamt of [1]. Nanotechnology has endowed humans with the capacity to understand, control, design and manipulate matter with the dexterity
of a craftsman at the simplest level of atoms and molecules. It is a technology that enables working with atoms and molecules that are sized on a nanometer (nm) scale with dimensions less than 100nm—these are the nanoparticles. The particles are being synthesized using different physical techniques such as lithography, laser ablation, sputtering deposition, pulsed electrochemical etching, vapor deposition, laser pyrolysis, plasma or flame spraying synthesis and chemical processes (sol gel processing). Despite the increasing awareness in production and use of nanoparticles in many consumer-based products, there is still growing interest in the biological and environmental safety of their production. These techniques suffer from a lot of setbacks including use of toxic solvents that are potentially hazardous to the environment and living organism [2,3], being labor intensive (consuming high energy) and very expensive [4,5]. The disadvantages of widely used physical and chemical processes have given rise to an alternative highly efficient, cost-effective and environmentally safe method of nanoparticles production; green synthesis.

2. Green Synthesis of silver nanoparticles and its mechanisms

Green synthesis is the art of synthesizing nanoparticles using biological systems which may include whole organisms (microbes, plants, and animals) or their metabolites; phytochemicals, protein and their derivatives; enzymes and templates like DNA or even membranes. Over the past decades, a lot of studies have demonstrated the synthesis of metal nanoparticles using different biological systems such as bacteria [6-8], fungi [9,10], plants and their metabolites [2-5,11-13], insects and their metabolites [14-17], cobweb [15], honey [16] and earthworm [18] to mention a few. A detailed analysis of different methods of green synthesis, biological systems used and the special attributes of the synthesized particles have been compiled [14].

Green synthesis of silver nanoparticles (AgNPs) usually involves suitable sources of biological system that have potential to elicit production of bioreductants, which manifest in form of enzymes or phytochemicals. Such bioreductants are known to be electron rich and therefore initiate the reduction of metal ions in solution to its nanoparticles (NPs). The reaction normally occur at ambient condition and the mix ratio of metal salt solution relative to the bioreductant in most cases is such that small quantity of bioreductant is added to large volume of
metal salt solution of low concentration \(1 \times 10^{-3} \text{ M}\) to initiate and effect subsequent formation of nanoparticles. For instance, in green synthesis of AgNPs, the ratio of bioreductant to metal salt solution (AgNO\(_3\)) by most researchers is usually around 1:40 \[13\] or 1:10 \[19\] with little variation. The process usually occur in two stages; (1) reduction stage in which the metal ions (Ag\(^+\)) are reduced to their nanoparticles (Ag\(^0\)) and (2) nucleation and stabilization stage in which each AgNP act as a nucleus surrounded by bioreductants to stabilize the AgNPs formed (Figure 1). By this later stage, the biogenic AgNPs synthesized can stay in storage without aggregation.

![Mechanisms of metal ions bioreduction to nanoparticles](image)

**Figure 1.** Mechanisms of metal ions bioreduction to nanoparticles \[20\]

When whole organisms (i.e. bacteria and fungi) are used in nanoparticles biosynthesis, the processes of metal ion bioreduction either occur outside the cell (extracellular accumulation of nanoparticles) or within the cell (cytoplasmic bioaccumulation of nanoparticles). In an extracellular accumulation of nanoparticles, membrane enzymes such as oxidoreductase or extracellular metabolites are produced which reduce metal ions to nanoparticles. The
nanoparticles produced are then obtained by simple purification. However, in cytoplasmic bioaccumulation of nanoparticles, enzymes or metabolites in the electron carrier system act as bioreductants that reduce metal ions to their nanoparticles (Figure 2). Such nanoparticles are then obtained by lysing the cell either mechanically or otherwise to harvest the nanoparticles produced.

**Figure 2.** Illustration of whole organism biosynthesis of metal nanoparticles

Many animal metabolites such as derivatives of protein, starch, fat and oil are known to be rich sources of bioreductants. In addition, many plant extracts are known to contain phytochemicals such as alkaloids and phenolic compounds of different classes that also serve as rich sources of bioreductants. These biomolecules in their crude forms or when purified have potential to reduce metal ions to their nanoparticles. The roles of these biomolecules are both as reducing and stabilizing agents. These mechanisms of green synthesis of nanoparticles are favoured over both physical and chemical routes due to their cheap, less time and energy demanding, non-toxic and ecofriendly nature. Aside, green synthesis that makes use of plant materials is the most preferred because an elaborate system of cell culture and maintenance is not required.
In the green synthesis of metal nanoparticles, the shape, size, optical, catalytic and biological properties of the synthesized nanoparticles turn to depend on a number of factors which include reactant concentrations, mix ratio, pH, reaction kinetics, solution chemistry, and interaction time among others. All these can be varied and manipulated with a view to synthesis nanoparticles of various sizes and forms for various purposes. For instance, when nanoparticles with size and structural uniformity are synthesized, they are said to be monodispersed and isotropic respectively, otherwise, they are polydispersed and anisotropic. Biosynthesized nanoparticles can be engineered by varying the reaction conditions to produce well-defined and uniform nanostructures as the situation demands (Figure 3). This also may offer an ample opportunity to scale up the bioprocess of metal nanoparticles production for wide applications in various fields of human endeavours.

**Figure 3.** Different forms and size of nanoparticles
3. Characterization of biogenic metal nanoparticles

Most green synthesized nanoparticles especially AgNPs are usually accompanied by colour change when initiated. This colour change varies from light yellow to pinkish through ruby red, faint blue to purple and finally to dark brown when it finally stabilized, which is mostly reported in literature [15, 21, 22, 23, 24, 25, 26] depending on shape and size of the particles. The excitation of the reduced particles is greatly influenced by the nature of the bioreductant molecules, the consequences of which lead to manifestation of different shades of colour. The synthesis of green nanoparticles is monitored with UV-visible spectrophotometer to investigate the plasma resonance band for metal nanoparticles at $\lambda_{\text{max}}$ in the range of 200-800 nm (Figure 4). For AgNPs, the display of surface plasmon resonance (SPR) band is usually at 390-460 nm [27, 28]. This is followed with Fourier Transform-Infrared (FTIR) spectroscopy to identify the specific functional groups present in the synthesized metal nanoparticles in the range of 400-4000 cm$^{-1}$ that might have played active roles in the reduction and capping of the nanoparticles. Scanning electron microscopy (SEM) is employed to study and unravel the surface topography of the particles usually expressed as function of shape and size (i.e. diameter) while energy dispersive spectroscopy is used to analyze the elemental constituents of the nanoparticles as facet of face-centered cubic lattice, while the transmission electron microscopy (TEM) analysis unravel the size and shape of the nanoparticles with selected area electron diffraction (SAED) to assess and discover the nature of the nanoparticles. X-Ray diffraction (XRD) analysis helps to confirm the crystalline nature of the nanoparticles.

![Figure 4. Typical UV-vis spectra for green synthesized AgNPs](image-url)
4. Homeostatic disorder in human

Blood clotting is the nature’s antidote to excessive bleeding. However, this portends a serious danger when such clot remains longer than necessary and obstructs blood flow in vessels supplying organs and tissues, resulting in clinical situations such as heart attacks and stroke. The formation of thrombus (a blood clot) in the circulatory system is part of the mechanism in human body that consolidates the repair of the injured or damaged blood vessel [29]. Consequent upon thrombus formation within blood vessels, the following clinical situations like embolism, ischemia, heart attack, stroke, and array of others may result [30].

Emboli is a phenomenon that precipitates blood clot formation in the circulatory system either within a vein or an artery, which may fully or partially impede free flow of blood to some parts of the body or body organs depending on its location. In such instance, a serious clinical situation may manifest with far reaching effects. For instance, occurrence of pulmonary embolism in which some arteries serving the lungs are blocked at the instance of embolus formation leads to inexplicable breathing difficulty, hemoptysis, and pain in and around the chest [31]. A blood clot that impedes free flow of blood or supply of oxygen to body tissues results in ischemia. A fully or partially restricted flow of blood to cardiac muscle otherwise called cardiac ischemia results in shortness of breath, syncope, angina, myocardial infarction, cardiac arrhythmia, or even death [32].

In addition, formation of blood clot in vessels innervating the brain tissues leads to an ischemic stroke as a consequence of restricted regular supply of blood to the brain [33]. Occurrence of ischemic stroke may be a direct consequences of restricted supply of blood to the brain (thrombolic stroke) due to blockage of blood vessel or alternatively, embolus produced elsewhere in the body from incidence of clot may be carried to some small arteries in the brain (embolic stroke). In some cases, blood clot initiated in the heart may find its way to the narrow arteries of the brain and create blockage that results in cerebral stroke. In whichever way it occurs, formation of blood clot in or around the blood vessels supplying the brain directly leads to starvation of brain tissue (i.e. of respiratory substrate), the consequences of which result in irreversible brain cell damage and death [34].
5. Anticoagulant and thrombolytic agents

Anticoagulants are agents that prevent and inhibit blood clot formation, whereas antiplatelet and thrombolytic agents are drugs used in reducing the incidence of blood clots or prevent existing clots from getting larger in the body. They are also called blood thinners. Drugs that interfere with blood coagulation (anticoagulants) are a main stay of cardiovascular therapy, of which atrial fibrillation, venous thromboembolism and artificial heart valves are the main indications for anticoagulation. An individual that is at risk of thrombotic condition is said to be prone to thromboembolism for each condition if not on anticoagulant therapy. Antithrombotic therapy is effectively used to manage atrial fibrillation closely connected to an increased risk of stroke and thromboembolic complications [35].

All over the world, venous thromboembolism (VTE) is the major cause of cardiovascular mortality, next in rank to myocardial infarction and stroke [36, 37]. This clinical situation affects patients in various settings and age groups, including the young children [38, 39]. The most prevalent manifestation of VTE is deep vein thrombosis (DVT), the expression of which may manifest in its most severe form known as acute pulmonary thromboembolism (PTE) [40]. Major treatment for both situations involves administration of full anticoagulation so as to minimize recurrence of VTE.

Over the years, it is a well-known and established fact that anticoagulation directly impacts or affects the mortality associated with VTE. The use of unfractionated or low-molecular-weight heparin and vitamin K antagonists, especially warfarin in the management of VTE was embraced and in use long before now. The traditional anticoagulants which were effective in the treatment of VTE have been found to be enshrouded with practical difficulties, necessitating the development and search for new classes of anticoagulants [40]. Two groups of new oral anticoagulant drugs such as factor Xa inhibitors (rivaroxaban, apixaban, and edoxaban) and direct thrombin inhibitors (dabigatran) have been reviewed for their efficacy and safety compared to the conventional or classical treatment using heparin and warfarin [40]. Despite their widespread use, they are still not without a number of shortcomings, prompting the need for development of an unprecedented number of new agents.

The classification, mechanism of action, route of administration and the attendant side effects or limitations of the various anticoagulants, antiplatelets and thrombolytics produced
synthetically were also reviewed [41, 42]. The side effects of the two drugs include bleeding (which may result to death if not managed properly), hemorrhage, osteoporosis, drug food reactions, drug induced hepatitis, alopecia, rashes and highly expensive cost. Traditional antithrombotic treatments like streptokinase also have limitations in their suitability for application. These may include short half-life, inactivation of the foreign agents by antibodies and possible incidence of excessive bleeding which may be life threatening. Nevertheless, application of liposomes that can resist degradation as carrier for streptokinase has greatly improved its thrombolytic activity [43]. Despite widespread use and efficiency, these anticoagulants still pose a threat to life and the problems besetting their administrations are still significant and are not easily discountenanced. Thus, there is need for development and design of new agents that are of biological origin, safe, ecofriendly with little or no side effects. The herald of nanotechnology has opened a window of opportunity for providing solution to these problems. This involves a process by which nontoxic and biocompatible nanoparticles are used as either stand-alone thrombolytic agents or carriers of active drugs [13].

6. Biogenic silver nanoparticles as anticoagulant and thrombolytic agents

Numerous and diverse metallic nanoparticles have been investigated, out of which AgNPs occupy prime position premised on their utility in several applications, which encompasses optical, electronic, catalytic, antimicrobial and electrochemical attributes. These have tremendously influenced and enhanced their relevance in vast areas of human endeavours such as food, healthcare, agriculture, biomedical, environmental, textile and catalytic applications [20]. Array of applications of AgNPs has remained endless as the improved compatibility with biological systems through avoidance of hazardous procedures and precursors are sought [13]. Of these new applications is an evolving and fast developing area of nanomedicine where biogenic AgNPs are employed and used to manage blood coagulation disorder.

It is clearly evident that nanoparticles can impart varying degree of influence on blood coagulation system, an attribute associated with their size, charge, shape and composition. They are also employed as carrier platform to redesign traditional drugs such as heparin with a potential to achieve reduced dosage, increased drug stability, shelf-life and overall cost benefit
delivery of treatment [44]. Most green synthesized AgNPs generally display surface plasmon band at 390 - 460 [10, 45], they are mostly crystalline, fairly spherical and polydispersed with size range of 4 - 95 nm, while very few are uniformly spherical and monodispersed with size range of 5-85 nm [19, 46, 47] (Table 1). Nanoparticles of gold [48] and iron oxides [49] have demonstrated the potential to serve as carriers of active drugs to reduce the limitations of short half-life, prohibitive cost, incidences of bleeding and hemorrhagic stroke as consequences of preventing blood coagulation. In the same vein, AgNPs have manifested antiplatelet activity to prevent aggregation of platelets [50] thereby inhibiting blood coagulation.

Some nanoparticles synthesized from various sources of biological materials and employed as both reducing and stabilizing agents (Table 1) have exhibited potential to serve as anticoagulants. Kim et al. [18] reported an improvement of 118.9% in the anticoagulant activities of heparin coupled with AuNPs biosynthesized using earthworm extract. Green synthesized gold and silver-gold alloy nanoparticles using cell-free extracts of Bacillus safensis LAU13 have been shown to exhibit anticoagulant and thrombolytic properties [6], while Kolanut extract-mediated silver-gold alloy nanoparticles have demonstrated thrombolytic potential [12]. In addition, AgNPs synthesized by the use of cell-free extracts [51], Cobweb, Kolanut pod, seed and seed shell extracts [45] also exhibited anticoagulants and thrombolytic activities.

Furthermore, paper wasp nest extract-mediated biosynthesized AgNPs manifested anticoagulant and thrombolytic activities [52] in which the preformed blood clots were dissolved instantaneously (Figure 5) whereas the negative control silver nitrate treated and nest extract-treated blood clots did not lead to lyses of the blood clots. In another study, cocoa bean extract-mediated AgNPs (CBE-AgNPs) was found to impede the coagulation of human blood in vitro without any distortion to biological properties and essential attributes of red blood cells (Figure 6) [2]. Fungal xylanases-mediated silver nanoparticles (NEA-AgNPs and TEA-AgNPs) were reported to inhibit clotting of fresh blood from healthy human donor while they also initiate dissolution of clot within 5 min when added to preformed blood clot [10]. Dakshayani et al. [19] reported antiplatelet activity along with anticoagulant effect of Selaginella bryopteris plant leaf extract-mediated AgNPs and inferred the usefulness of the nanoparticles in thrombus disorder treatment, while the work of Raja et al. [46] further confirmed the utility of biogenic AgNPs as
Table 1. Some green synthesized nanoparticles with anticoagulant and thrombolytic properties

| Biological resource | Particles synthesized | Organisms or part used | Size and special features | Reference |
|---------------------|-----------------------|------------------------|--------------------------|-----------|
| Bacteria; *Bacillus safensis* | Ag-Au | Cell-free extract | fairly spherical; 5-95 nm | [6] |
| Bacteria; *B. safensis* | Ag | Cell-free extract | Fairly spherical; 5-95 nm | [45] |
| Fungi; *Aspergillus niger* L3 and *Trichoderma longibrachiatum* L2 | Ag, Au & Ag-Au | Crude xylanases extract | Spherical, cylindrical to oval; 15.21-77.49 nm for AgNPs, spherical and flower shaped; 4.88-123.99 nm for AuNPs, spherical, oval to irregular; 6.98-52-51 nm | [2, 10, 57, 58] |
| Insect; *Polistes sp* | Ag | Paper wasp nest | crystalline polydisperse, 12.5-95.55 nm, Anisotropic structure of sphere, triangle, hexagon, rod and rhombus | [14] |
| Insect; Spider | Ag | Cobweb; | Nearly spherical, polydispersed with size ranging 3-50 nm, | [15] |
| Earthworm; *Eisenia andrei* | Au | Cell-free extract | 6.13 nm in size, spherical, cubic and block shaped | [18] |
| Plant; *Synsepalum dulcificum* | Ag | Leaf extract | 5-22 nm well dispersed | [13] |
| S. dulcificum | Ag | Seed extract | 4-26 nm, spherical and crystalline in nature with little agglomeration | [13] |
| *Cola nitida* | Ag-Au | Leaf extract | polydispersed, near spherical Size range 17-90 nm | [12] |
| *Cola nitida* | Ag-Au | Seed extract | polydispersed, near spherical 17-90 nm | [12] |
| *Theobroma cacao* | Ag | Seed extract | 8.96-54.22 nm, well dispersed and spherical | [2] |
| *Cola nitida* | Ag-Au | Seed shell | polydispersed, near spherical 17-90 nm | [6] |
| *Cola nitida* | Ag-Au | Pod extract | anisotropic mixture of near spherical, hexagonal, rod and triangular particles with size 12-91 nm | [6] |
| Plant: *Cola nitida* | Ag | Pod, Seed and Seed shell | Spherical, polydispersed with size 12-80 nm, 8-50 nm and 5-40 nm respectively | [12, 13] |
| *Selaginella bryopteris* | Ag | Leaf extract | Crystalline, fairly spherical and monodispersed, uniform distribution with size 5-10 nm | [19] |
| *Peltophorum pterocarpum* | Ag | Pods | Crystalline, uniformly spherical shape with size 70-85 nm | [46] |
| *Petiveria alliacea* | Ag | Leaf extract | Crystalline monodispersed with size range 16.70-33.74nm | [47] |
| *Chasmanthera dependens* | Ag | Stem extract | Cubic; 24.53-92.38 nm | [59] |
Figure 5. Anticoagulant (a), and thrombolytic activities (b, c) of biosynthesized AgNPs (FB; Fresh blood, EDTA; Anticoagulant, WHA; Wasp nest extract AgNPs, BC; Blood clot, S; AgNO₃ solution, WH; Wasp nest extract only) [48]

Figure 6. Anticoagulant activity of CBE-AgNPs [2]
anticoagulating agent by treating freshly collected blood with *Pe ltophorum pterocarpum* plant pods mediated AgNPs. Most of cited studies suggested nontoxic nature of green AgNPs to platelets and this coupled with established actions against microbes represent a paradigm shift in preventing blood coagulation. These evidences further underscore the potential of biogenic AgNPs to manage blood disorder. Lateef *et al.* [53] previously reviewed the contributions of nanoparticles for the management of blood coagulation disorders.

7.0 Proposed mechanism of action for thrombolysis by green AgNPs

AgNPs may be capable of inhibiting fibrinogen to prevent fibrin formation and consequently prohibiting blood coagulation. The whole events take place in a complex system involving series of clotting factors in which AgNPs might have acted in such a way to inhibit factors II, VII and X to prevent thrombin formation [2]. However, this is not yet fully understood but notwithstanding, the combined antiplatelet, fibrinolytic and possible fibrinogen-inhibiting activities undoubtedly play an active role in the anticoagulant activity of AgNPs. Two mechanisms were proposed: (1) that AgNPs probably act on plasminogen resulting in its activation to evoke the release of plasmin which then acts on the blood clot and breaks it or (2) that AgNPs may directly act on fibrin and cause it to break down as presented by Harish *et al.* [54]. In addition to mechanism 1, the nanoparticles may inhibit the activities of the inhibitors such that activation of plasminogen and plasmin may be prevented. The two mechanisms may also occur concurrently leading to pronounced thrombolytic activities as obtained in most studies (Figure 7).

8. Challenges of green silver based anticoagulant and thrombolytic agents

Biogenic AgNPs have clearly exhibited anticoagulant and thrombolytic activities with potential for biomedical applications. Of importance is the application of biologically synthesized AgNPs in this area of healthcare delivery where nontoxic and biocompatibility features are most desirable. However, some studies have highlighted the possibility of some green synthesized AgNPs not to be completely nontoxic and biocompatible as they have demonstrated cytotoxic, genotoxic and mito-depressive activities on the cells of *Allium cepa* roots [55, 56]. This safety concern and other number of hurdles have to be overcome before
biogenic silver drug can be deployed for use in healthcare system. Most investigations on toxicity and compatibility were conducted in vitro, thus implying the need to explore in vivo investigations on potential biosynthesized AgNPs-based anticoagulants. In addition, the biochemical interaction with body cells and final fate of the metallic silver after the anticoagulation and thrombolytic events are yet to be fully understood. Moreover, the chemistry of action or pharmacokinetics of biogenic AgNPs should also be unraveled. Successful execution of these studies should be followed by design of potential AgNPs-based drugs for clinical trials. Nevertheless, the existing evidences here would be needed for effective and safe use of biogenic AgNPs as anticoagulant and thrombolytic agents in the immediate future.

**Figure 7.** The proposed mechanisms for thrombolytic activities of biogenic AgNPs [13]

### 9. Conclusion

There are clear and unambiguous evidences that AgNPs have anticoagulant and thrombolytic potentials. Biosynthesized AgNPs have also demonstrated not only anticoagulant and thrombolytic potentials but also possibility of suitability in healthcare delivery because of
implied nontoxic and biocompatible nature. Furthermore, probable modes of action of these nanoparticles have been proposed. However, the anticoagulant and thrombolytic potentials of biogenic nanoparticles are still underexploited due to limited reports in literature. There is therefore need for more intensive research effort on biologically synthesized metal nanoparticles with the view of exploring their application in biomedicine, most especially in the management and subsequent treatment of blood disorders. Also, the mechanism of action of these bio-nano agents coupled with their pharmacokinetics should be unraveled and well established. This becomes very important in a bid to ascertain their safe administration in healthcare delivery compare to the chemically synthesized anticoagulant and thrombolytic agents.

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References

[1] Preeti NJ 2016 Green chemistry for nanotechnology: Opportunities and future challenges. Res. Rev. Chem. 5 (1) pp 2319-9849.

[2] Azeez MA, Lateef A, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Gueguim-Kana EB and Beukes LS 2017 Biomedical applications of cocoa bean extract-mediated silver nanoparticles as antimicrobial, larvicidal and anticoagulant agents. J. Clust. Sci. 28 pp 149-164.

[3] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Azeez L, Ojo SA, Gueguim-Kana EB and Beukes LS 2016 Cocoa pod husk extract-mediated biosynthesis of silver nanoparticles: its antimicrobial, antioxidant and larvicidal activities. J. Nanostruct. Chem. 6 (2) pp 159-69.

[4] Shankar SS, Rai A, Ahmad A and Sastry M 2004 Rapid of Au, Ag and bimetallic Au core – Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J. Colloid Interf. Sci. 275 (2) pp 496-502.
[5] Philip D 2011 *Mangifera indica* leaf-assisted biosynthesis of well-dispersed silver nanoparticles. *Spectrochim. Acta. A.* **78** (1) pp 327-331.

[6] Ojo SA, Lateef A, Azeez MA, Oladejo SM, Akinwale AS, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Gueguim-Kana EB and Beukes LS 2016 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.* **15** (5) pp 433-42.

[7] Narayanan KB and Sakthivel N 2010 Biological synthesis of metal nanoparticles by microbes. *Adv. Colloid Interf. Sci.* **156** (1-2) pp 1-13.

[8] Lengke MF, Fleet ME and Southam G 2007 Biosynthesis of silver nanoparticles by filamentous cyanobacteria from nitrate complex. *Langmuir* **23** (5) pp 2694-2699.

[9] Rautaray D, Ahmad A and Sastry M 2003 Biosynthesis of CaCO$_3$ crystals of complex morphology using fungus and an actinomycete. *J. Am. Chem. Soc.* **125** (48) pp 14656-14657.

[10] Elegbede JA, Lateef A, Azeez MA, Asafa TB, Yekeen TA, Oladipo IC, Adebayo EA, Beukes LS and Gueguim-Kana EB 2018 Fungal xylanases-mediated synthesis of silver nanoparticles for catalytic and biomedical applications. *IET Nanobiotechnol.* **12** (6) pp 857-863.

[11] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, Azeez L, Ojo SA, Gueguim-Kana EB and Beukes LS 2016 Cocoa pod husk extract-mediated biosynthesis of silver nanoparticles: Its antimicrobial, antioxidant and larvicidal activities. *J. Nanostruct. Chem.* **6** (2) pp 159-169.

[12] Lateef A, Ojo SA, Folarin BI, Gueguim-Kana EB and Beukes LS 2016 Kolanut (*Cola nitida*) mediated synthesis of silver-gold alloy nanoparticles: antifungal, catalytic, larvicidal and thrombolytic applications. *J. Clust. Sci.* **27** pp 1561-1577.

[13] Lateef A, Akande MA, Azeez MA, Ojo SA, Folarin BI, Gueguim-Kana EB and Beukes LS 2016 Phytosynthesis of silver nanoparticles (AgNPs) using miracle fruit plant (*Synsepalum dulcificum*) for antimicrobial, catalytic, anticoagulant, and thrombolytic applications. *Nanotechnol. Rev.* **5** (6) pp 507-520.
[14] Lateef A, Ojo SA and Elegbede JA 2016 The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles. *Nanotechnol. Rev.* 5 (6) pp 601-622.

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

[16] Eranga RB, Chanika DJ, Uthpala AJ, Ranasinghe WD, Ruwanthika RM and Pretti VU 2017 Honey mediated green synthesis of nanoparticles: New era of safe Nanotechnology. *J. Nanomater.* https://doi.org/10.1155/2017/5919836.

[17] Ghosh S, Patil S, Ahire M, Kittere R, Gurav DD, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare J, Dhavale DD and Chopade BA 2012 *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. *J. Nanobiotechnol.* 10 17. https://doi.org/10.1186/1477-3155-10-17.

[18] Kim HK, Choi M, Cha S, Koo YK, Jun SH, Cho S and Park Y 2013 Earthworm extracts utilized in the green synthesis of gold nanoparticles capable of reinforcing the anticoagulant activities of heparin. *Nanoscale Res. Lett.* 8 542. https://doi.org/10.1186/1556-276X-8-542.

[19] Dakshayani SS, Marulasiddeshwara MB, SharathKumar MN, Ramesh G, Kumar PR, Devaraja S and Hosamani R 2019 Antimicrobial, anticoagulant and antiplatelet activities of green synthesized silver nanoparticles using *Selaginella* (Sanjeevini) plant extract. *Int. J. Biol. Macromol.* https://doi.org/10.1016/j.ijbiomac.2019.01.222.

[20] Keat CL, Aziz A, Eid AM and Elmarzugi NA 2015 Biosynthesis of nanoparticles and silver nanoparticles. *Bioresour. Bioprocess.* 2 47. https://doi.org/10.1186/s40643-015-0076-2.

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

[22] Lateef A, Adelere IA, Gueguim-Kana EB, Asafa TB and Beukes LS 2015 Green synthesis of silver nanoparticles using keratinase obtained from a strain of *Bacillus safensis* LAU 13. *Int. Nano Lett.* 5 pp 29-35.
[23] Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinbورو A, Oladipo IC, Ajetomobi FE, Gueguim-Kana EB and Beukes LS 2015 Cola nitida mediated biogenic synthesis of silver nanoparticles using seed and seed shell extracts and evaluation of antibacterial activities. *BioNanoSci.* **5** pp 196-205.

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

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

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

[27] He Y, Wei F, Ma Z, Zhang H, Yang Q, Yao B et al. Green synthesis of silver nanoparticles using seed extract of *Alpinia katsumadai*, and their antioxidant, cytotoxic, and antibacterial activities. *RSC Adv.* **7** (63) pp 39842-39851.

[28] Das G, Patra JK, Debnath T, Ansari, A, Shin H-S 2019 Investigation of antioxidant, antibacterial, antidiabetic, and cytotoxicity potential of silver nanoparticles synthesized using the outer peel extract of *Ananas comosus* (L). *PLoS ONE* **14** (8) E0220950.

[29] Lillicrap D, Key N, Makris M and O’Shaughnessy D 2010 Practical Hemostasis and Thrombosis 3rd Edition. *Wiley-Blackwell Publishing Ltd*. 311pp.

[30] Shapiro SS 2003 Treating thrombosis in the 21st century. *The New Engl. J. Med.* **349** (18) pp 1762-1764.

[31] Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL and Jameson JL 2005 Harrison’s Principles of Internal Medicine, 16th ed. *McGraw-Hill, Medical Publishing Division NY*. **2** 607pp.

[32] Maseri A, Severi S, Nes MD 1978 Variant angina: one aspect of a continuous spectrum of vasospastic myocardial ischemia; Pathogenetic mechanisms, estimated incidence and
clinical and coronary arteriographic findings in 138 patients. *Am. J. Cardiol.* **42** (6) pp 1019-1035.

[33] Shiber JR, Fontane E and Adewale A 2010 Stroke registry: hemorrhagic vs ischemi strokes. *Am. J. Emerg. Med.* **28** (3) pp 331-333.

[34] Ohira T, Shahar TE, Chambless LE, Rosamond WD, Mosley TH and Folsom AR 2006 Risk factors for ischemic stroke subtypes: the atherosclerosis risk in communities study. *Stroke* **37** (10) pp 2493-2498.

[35] Thachil J, Gatt A and Martlew V 2008 Management of surgical patients receiving anticoagulation and antiplatelet agents. *Brit. J. Surg.* **95** pp 1437-1448.

[36] Cohen AT, Agnelli G, Anderson FA, Arcelus JI, Bergqvist D and Brecht JG 2007 Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. *Thromb. Haemost.* **98** (4) pp 756-764.

[37] Heitt JA 2008 The epidemiology of venous thromboembolism in the community. *Arterioscl. Thromb. Vascul. Biol.* **28** (3) pp 370-372.

[38] Biss TT, Brandão LR, Kahr WH, Chan AK and Williams S 2008 Clinical features and outcome of pulmonary embolism in children. *Brit. J. Haematol.* **142** (5) pp 808-818.

[39] Andrade Ede O, Bindá FA, Silva AM, Costa TD and Fernandes MC 2009 Risk factors and prophylaxis for venous thromboembolism in hospitals in the city of Manaus. *J. Brasileiro de Pneumol.* **35** (2) pp 114-121.

[40] Santos Fernandes CJS, AlvesJúnior JL, Gavilanes F, Prada LF, Morinaga LK and Rogerio S 2016 New anticoagulants for the treatment of venous thromboembolism. *J. Brasileiro de Pneumol.* **42** (2) pp 146-154.

[41] Ramjan A, Hossain MS, Ariful Islam A, Arman SI, Raju GS, Dasgupta P, Noshin TF 2014 Aspect of thrombolytic therapy: A review. *Sci. World J.* Article ID 586510. [https://doi.org/10.1155/2014/586510](https://doi.org/10.1155/2014/586510).

[42] Jhansi K and Vanita P 2014 A Review on Antiplatelet Drugs and Anticoagulants. *Adv. Pharmacoepidemiol. Drug Safety* **3** (3-R). [https://doi.org10.4172/2167-1052.1000003-R](https://doi.org10.4172/2167-1052.1000003-R).

[43] Vaidya B, Agrawal GP and Vyas SP 2011 Platelets directed liposomes for the delivery of streptokinase: development and characterization. *Eur. J. Pharmaceut. Sci.* **44** pp 589-594.
[44] Ilinskaya AN and Dobrovolskaia MA 2013 Nanoparticles and the blood coagulation system Part I: benefits of nanotechnology. *Nanomed.* 8 pp 773-784.

[45] Lateef A, Ojo SA, Elegbede JA, Azeez MA, Yekeen TA and Akinboro A 2017 Evaluation of some biosynthesized silver nanoparticles for biomedical applications: hydrogen peroxide scavenging, anticoagulant and thrombolytic activities. *J. Clust. Sci.* 28 (3) pp 1379-1392.

[46] Raja S, Ramesh V and Thivaharan V 2015 Antibacterial and anticoagulant activity of silver nanoparticles synthesised from a novel source-pods of *Peltophorum pterocarpum*. *J. Clust. Sci.* 28 (3) pp 1379-1392.

[47] Lateef A, Folarin BI, Oladejo SM, Akinola PO, Beukes LS and Gueguim-Kana EB 2018 Characterization, antimicrobial, antioxidant, and anticoagulant activities of silver nanoparticles synthesized from *Petiveria alliacea* L. leaf extract. *Prep. Biochem. Biotechnol.* 48 (7) pp 646-652.

[48] Shiang YC, Hsu CL, Huang CC and Chang HT 2011 Gold nanoparticles presenting hybridized self-assembled aptamers that exhibit enhanced inhibition of thrombin. *Angew. Chem.* 123 (33) pp 7802-7807.

[49] Hsu CL, Chang HT, Chen CT, Wei SC, Shiang YC and Huang CC 2011 Highly efficient control of thrombin activity by multivalent nanoparticles. *Chem.* 17 pp 10994-11000.

[50] Shrivastava S, Bera T, Singh SK, Singh G, Ramachandrarao P and Dash D 2009 Characterization of antiplatelet properties of silver nanoparticles. *ACS Nano.* 3 (6) pp 1357-1364.

[51] Lateef A, Ojo SA and Oladejo SM 2016 Anti-candida, anti-coagulant and thrombolytic activities of biosynthesized silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13. *Process Biochem.* 51 (10) pp 1406-1412.

[52] Lateef A, Akande MA, Ojo SA, Folarin BI, Gueguim-Kana EB and Beukes LS 2016 Paper wasp nest-mediated biosynthesis of silver nanoparticles for antimicrobial, catalytic, anticoagulant, and thrombolytic applications. *3Biotech.* 6 (2) pp 140. [https://doi.org/10.1007/s13205-016-0459-x](https://doi.org/10.1007/s13205-016-0459-x).

[53] Lateef A, Ojo SA, Elegbede JA, Akinola PO and Akanni EO 2018 Nanomedical applications of nanoparticles for blood coagulation disorders. In: *Environmental*
Nanotechnology, Volume 1. Eds: Dasgupta N, Ranjan S and Lichtfouse E. https://doi.org/10.1007/978-3-319-76090-2_8. Springer International Publishing AG, Cham, Switzerland. ISBN 978-3-319-76089-6. Pp. 243-277.

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

[55] Yekeen TA, Azeez MA, Akinboro A, Lateef A, Asafa TB, Oladipo IC, Oladokun SO and Ajibola AA 2017 Safety evaluation of green synthesized Cola nitida pod, seed and seed shell extracts-mediated silver nanoparticles (AgNPs) using an Allium cepa assay. J. Taibah Univ. Sci. 11 (6) pp 895-909.

[56] Yekeen TA, Azeez MA, Lateef A, Asafa TB, Oladipo IC, Badmus JA, Adejumo SA and Ajibola AA 2017 Cytogenotoxicity potentials of cocoa pod and bean-mediated green synthesized silver nanoparticles on Allium cepa cells. Caryol. 70 (4) pp 366-377.

[57] Elegbede JA, Lateef A, Azeez MA, Asafa TB, Yekeen TA, Oladipo IC, Aina DA, Beukes LS and Gueguim-Kana EB 2020 Biofabrication of gold nanoparticles using xylanases through valorization of corncob by Aspergillus niger and Trichoderma longibrachiatum: antimicrobial, antioxidant, anticoagulant and thrombolytic activities. Waste Biomass Valor. 11 (3) pp 781-791.

[58] Elegbede JA, Lateef A, Azeez MA, Asafa TB, Yekeen TA, Oladipo IC, Abbas SH, Beukes LS and Gueguim-Kana EB 2019 Silver-gold alloy nanoparticles biofabricated by fungal xylanases exhibited potent biomedical and catalytic activities. Biotechnol. Progr. 35 e2829. https://doi.org/10.1002/btpr.2829.

[59] Aina DA, Owolo O, Lateef A, Aina FO, Abbas SH, Adeoye-Isijola M, Okon V, Asafa TB, Elegbede JA, Olukanni OD and Adediji I 2019 Biomedical applications of Chasmanthera dependens stem extract mediated silver nanoparticles as antimicrobial, antioxidant, anticoagulant, thrombolytic, and larvicidal agents. Karbala Int. J. Modern Sci. 5 (2) pp 71-80.