Synthesis, bioactivities and cytogenotoxicity of animal fur-mediated silver nanoparticles

G O Akintayo, A Lateef, M A Azeez, T B Asafa, I C Oladiipo, J A Badmus, S A Ojo, J A Elegbede, E B Gueguim-Kana, L S Beukes and T A Yekeen

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

6Department of Microbiology, 7Microscopy and Microanalysis Unit, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

8Nanotechnology Research Group (NANO+) (www.nanotech.lautech.edu.ng)

*Corresponding author: tayekeen@lautech.edu.ng

Abstract

Animal waste materials are rarely used in the synthesis of nanoparticles compared to microorganisms and plant materials. The use of animal fur (goat) in synthesis could assist in turning waste to wealth. Thus, potentials of animal fur in the synthesis of silver nanoparticles (AF-AgNPs), its biological activities and safety through cytogenotoxicity were investigated. Animal fur (1 g) was hydrolyzed with 100 ml of 0.1 M NaOH at 90 °C for 1 h, cooled and centrifuged at 4000 rpm for 30 min. The extract (1 ml) was added to 1 mM AgNO₃ (40 ml) to reduce Ag⁺ to its nanoparticles. The AF-AgNPs was characterized using UV–vis spectroscopy, Fourier-transform-infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and Energy Dispersive X-ray (EDX) analysis. Larvicidal, antioxidant, anticoagulant and thrombolytic potentials of AF-AgNPs were studied. Onion bulbs (20) were exposed to 0.01, 0.10, 1.0, 10.0 and 100.0 µg/ml of AF-AgNPs solution for its cytogenotoxicity study with AgNO₃ solution and distilled water as controls. Microscopic (24, 48 and 72 h) assessment of the onion cells and macroscopic (72 h) evaluation of the roots were also studied. The AF-AgNPs solution was brownish with surface plasmon resonance at 419 nm. Evaluation of FTIR spectra...
showed that protein molecules were used as capping and stabilization agents. The AF-AgNPs had size range of 11.67-31.47 nm, caused 60-100% mortality of exposed *Anopheles* mosquito larvae in 12 h, and scavenged DPPH (40-59%) and hydrogen peroxide (75-94%). The nanoparticles also exhibited anticoagulant and thrombolytic potentials on human blood with 25% lysis compared to 13% observed for only extract. Various chromosomal aberrations and growth inhibition were induced by AF-AgNPs especially at 72 h of 100 µg/ml. Extract from animal fur was explored in biogenic synthesis of nanoparticles and found to have high potentials as antioxidant, anticoagulant, thrombolytic agents. Inhibition of cell growth observed especially at highest concentration can be explored in anticancer drugs though with caution due to AF-AgNPs potential to induce chromosomal aberrations.

1 Introduction

Nanoparticles are found useful in different facet of human life with applications in pharmaceutics, catalysis, cosmetics, food packaging, medical devices, textiles, electronics, optics, fuel cells, biosensors, water treatment technology and in agriculture [1-4]. Green synthesized nanoparticles are mostly from micro-organisms and plant materials. Microorganisms including bacteria [5, 6] and fungi [7-9] as well as various plant materials [10-13] had been reported to mediate the synthesis of various nanoparticles. The extracts from various materials used in the synthesis of nanoparticles have been found to possess biomolecules that serve as reducing and capping agents.

In the biosynthesis of nanoparticles, animal materials and their metabolites are seldom used despite the fact that they possess biomolecules that can serve as reducing and capping agents. Reported cases of biosynthesis involving the use of animal materials include that of cobweb, paper wasp net, and cockroaches wings [14-16]. Further more, honey from bees (insect) had been reported to mediate green synthesis of gold, silver, carbon, platinum, and palladium nanoparticles by acting as both stabilizing and reducing agents as well as a precursor in nanoparticle synthesis [17]. Cow milk had also been reported in the synthesis of AgNPs and with potency as antifungal agent [18]. The proteins present in these biological materials are involved in the reduction and stabilization of nanoparticles so produced [19].
Quite a lot of animal waste products are found in the environment that constitute nuisance. These include animal fur, human hair, and chicken feathers among others. Employing these materials in the green synthesis will assist in turning waste to wealth and while the benefit that could be derived from the nanoparticles will still be achieved. This study sought to use animal fur (goat fur) in the synthesis of AgNPs and evaluate its bioactivities and cytogenotoxicity.

2 Materials and Methods

2.1 Experimental materials
Sample of animal fur (goat) were obtained from a local farmer in Ogbomoso, while the larvae of Anopheles mosquito were collected from stagnant water and were prepared for the experiment.

2.2 Green Synthesis of silver nanoparticles
The fur extract was made by adopting the modified method of Tszydel et al. [20] by hydrolyzing 1 g in 100 ml of 0.1M NaOH at 90 °C for 1 h. The hydrolyzed fur was cooled and centrifuged at 4000 rpm for 30 min and supernatant was obtained. This extract was used to synthesize AgNPs as previously described by Lateef et al. [21] where 1 ml of the extract was added to the reaction vessel containing 40 ml of 1 mM silver nitrate (AgNO₃) solution for the reduction of silver ion to nano size. The reaction was carried out for 15 min under bright condition for photoactivation. The formation of AgNPs was monitored through visual observation of change in colour (Figure 1) as described by Morones et al. [22].

2.3 Characterizations of the synthesized AgNPs
The AgNPs synthesized through the fur extract was characterized using UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy, Transmission electron microscopy (TEM), and Energy Dispersive X-ray (EDX).

2.4 Applications of synthesized nanoparticles
2.4.1 Antioxidant activities
DPPH free radicals scavenging assay was carried out using the modified method of Williams et al. [23] where 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of the AF-AgNPs was determined with different concentrations (1, 2, 5, 10, 20 and 40 µg/ml) of the nanoparticles. The antioxidant activity was determined by adding 1 ml of AF-AgNPs to 3.0 ml of
DPPH solution, and then kept in a dark room for 30 min before finally read at 517 nm with spectrophotometer (Shimadzu, UK).

\[
\% \text{DPPH scavenging effect} = \frac{AC - AS}{AC} \times 100
\]

Where: AC= absorbance of negative control and AS= absorbance of sample.

Hydrogen peroxide scavenging assay was evaluated using the modified method of Nabavi et al. [24, 25]. A solution of H$_2$O$_2$ (40 mM) was prepared in phosphate buffer of pH 7.4. The concentration of H$_2$O$_2$ was determined by absorption using spectrophotometer. A 4 ml of different concentrations of 1, 2, 5, 10, 20, 40, 60 and 80 µg/ml of AF-AgNPs and distilled water was added to 0.6 ml, 40 mM of H$_2$O$_2$ solution. The absorbance of H$_2$O$_2$ at 610 nm was determined after 20 min against a blank solution (phosphate buffer with H$_2$O$_2$).

\[
\% \text{H}_2\text{O}_2 \text{ scavenging effect} = \frac{AC - AS}{AC} \times 100
\]

Where: AC= absorbance of negative control and AS= absorbance of sample

2.4.2 Anticoagulant activity

The anticoagulant activity was performed by mixing 0.5 ml of 150 µg/ml AF-AgNPs with 5 ml of freshly collected human blood from an adult volunteer. This was held at ambient condition to observe coagulation of blood. A control experiment was set up for comparison [26].

2.4.3 Thrombolytic activity

The thrombolytic assay was performed by recording the weight of Eppendorf tube (X$_1$), dispensed 0.5 ml of blood into the tube then allowed to stand for 30 to 45 min for clot formation at room temperature of 37 °C (X$_2$). The weight of the clot was calculated as A=X$_2$-X$_1$. The AF-AgNPs (100µl) was added to the blood clot formed and incubated at room temperature of 37 °C for 90 min. The free fluid generated was removed and the weight was recorded again (X$_3$) while the weight of clot left was calculated as B=X$_3$-X$_1$. Percentage clot lysis was calculated as:

\[
\frac{A - B}{A} \times 100
\]

Finally, the microscopic analysis of the released fluid was performed using x40 objective lens [12, 27].

2.4.4 Larvicidal activity of AF-AgNPs

The AF-AgNPs was subjected to a dose-response bioassay for larvicidal activity against Anopheles mosquito’s larvae using the method of Bansal et al. [28]. Equal volume (10 ml) of different AgNPs concentrations (10, 20, 40, 60, 80 and 100 µg/ml) was used for the toxicity test
against 10 Anopheles mosquito larvae in triplicates. The percentage mortality rates were determined at 12 h interval for 36 h.

2.5 Allium cepa assay
Two hundred and fifty (250) onion bulbs (Allium cepa) were purchased at Wazo market in Ogbomoso and sun dried for two weeks. The outer scales of the bulbs and dried roots were carefully removed leaving the root primordial intact. Five concentrations i.e. 0.01, 0.1, 1.0, 10 and 100 µg/ml of AF-AgNPs, salt solution of silver and extract alone were prepared from the stock solutions and distilled water was used as diluent as well as negative control. Twenty onion bulbs were planted per concentration and were grown in dark cupboard. The test solutions were changed every 24 h to supply fresh nutrient to the growing bulbs. At 24, 48 and 72 h, 5 out of the onion bulbs planted for each concentration and controls were harvested and thoroughly rinsed in distilled water and fixed in ethanol: glacial acetic acid (3:1 v/v) in sample bottles per onion bulb, and samples were kept in the refrigerator. Already fixed and refrigerated root tips (2 or 3) were picked and hydrolyzed in 1N HCl at 65 °C for 3 min. The meristematic region of the root tip of two roots was cut on a clean glass slide and macerated using needle. A drop of aceto-orcein stain was added and the tissue on the slide was allowed to stain for 10 min, followed by scoring under light microscope as previously described [29-32]. At 72 h, roots of 5 bulbs per concentration and controls were harvested to evaluate growth inhibition.

2.6 Statistical analysis
Data were analyzed using SPSS 20.0. The mean root lengths obtained for the treated bulbs at different concentrations were compared using one way analysis of variance and Duncan multiple range test as post hoc test. Mitotic index, mitotic inhibition and percentage aberrant cells were calculated as follow:

\[ \text{Mitotic Index MI} = \frac{\text{number of dividing cells in treated } A.\text{cea}}{\text{total number of cells counted}} \times 100 \]

\[ \text{Mitotic Inhibition} = \frac{\text{MI of negative control } - \text{MI of treated}}{\text{MI of negative control}} \times 100 \]

\[ \% \text{ aberration per total cells scored} = \frac{\text{number of aberrant cells}}{\text{total number of cells scored}} \times 100 \]
3 Results and Discussion

3.1 Green synthesized AgNPs

The animal fur mediated the synthesis of AgNPs within 8-10 min of reaction in the vessel under ambient conditions of room temperature (30 ± 2 ºC) with a brown colouration, which further intensified with time but stabilized within 15 min of exposure to sunlight (Figure 1). A number of authors have reported variations in the colour of AgNPs colloidal solutions due to the composition of bioreductant responsible for the synthesis of the nanoparticles. Lateef et al. [21] reported the formation of dark brown AgNPs solution using the crude extracellular keratinase of strain of B. safensis LAU 13, Jeevan et al. [33] reported formation of yellowish brown AgNPs using cultured supernatant of Pseudomonas aeruginosa, while Oladipo et al. [6] reported dark brown AgNPs using cell free-extracts of Enterococcus species.

![Figure 1. Green synthesis of AF-AgNPs from fur extract and its UV-visible spectrum](image-url)
3.2. Characterization of biosynthesized AgNPs

The UV-vis spectrum of biosynthesized AF-AgNPs had broad absorbance peak at the wavelengths of 419 nm (Figure 1) which was within the range of 391-440 for AgNPs [18, 31]. The broad peak is an indication that the biosynthesized AF-AgNPs were polydispersed, observations similar this had been reported for various metal nanoparticles [33, 35].

The FTIR absorption spectrum showed distinct peaks at 3784, 3309, 1635 cm$^{-1}$ for the animal fur (Figure 2). The FTIR-spectra showed the transmittance of different chemical functional groups present in the fur extract treated with AgNO$_3$. The bands 3784 and 3309 cm$^{-1}$ are typical of H bonded -OH and –OH inter H bond, respectively while 1635 cm$^{-1}$ represents alkenyl C=C stretch. These indicate that proteins were the capping and stabilization biomolecules involved in the synthesis of AgNPs [36].

![Figure 2. The FTIR spectrum of the animal fur-mediated AgNPs](image)

The TEM micrograph revealed that the AF-AgNPs were spherical in shape with size ranging from approximately 11 to 32 nm (Figures 3A). These observations are similar to earlier reports described by Kannan et al. [37] where well dispersed particles were obtained. This indicates good stability of AF-AgNPs devoid of aggregation. The EDX pattern (Figure 3B) showed the predominance of silver in the AgNPs solution and is characterized with ring-like SAED pattern.
(Figure 3C) typical of the face-centered cubic crystalline structure of silver [15, 38]. It can be deduced that animal fur extract that mediated the synthesis of AgNPs is a novel material for the biogenic and ecofriendly synthesis.

![TEM micrograph, EDX spectrum, and SAED of AF-AgNPs](image)

**Figure 3.** TEM micrograph (A), EDX spectrum (B) and SAED (C) of AF-AgNPs

### 3.3 Antioxidant activities

The DPPH-scavenging activities of the nanoparticles are presented in Figure 4. The synthesized AF-AgNPs showed inhibitions of 40-59 % while ascorbic acid used as the standard produced inhibitions of 41-60 % at same concentrations ranging from 1 to 40 µg/ml. The result of antioxidant activities of biosynthesized AgNPs showed significant free radical scavenging activities when compared with the standard ascorbic acid, which is similar to other reports [6, 7]. The hydrogen peroxide scavenging activities also displayed dose dependent reduction activities of 75-94 %, at concentrations of 1–80 µg/ml (Figure 4). The scavenging activities of the AgNPs observed in this work are similar to those that were previously reported [12, 39]. The free radical scavenging activity of the AgNPs has been credited to the functional groups of the biomolecules which adhered to the surface of the nanoparticles. Also, various researchers have proposed that antioxidants repress and inhibit damages caused via oxidative stress to components of the cell
such as proteins, lipids and DNA which thus reduce the risk of chronic diseases related to aging [40].

![Antioxidant activities of AF-AgNPs](image)

**Figure 4.** Antioxidant activities of AF-AgNPs

### 3.4 Anticoagulant activities of biosynthesized AgNPs

The AgNPs prevented formation of blood clot when used as an anticoagulation agent, which is comparable to result obtained with EDTA. The microscopic examination of both EDTA and AF-AgNPs treated blood samples showed typical disc-shaped red blood cells (Figure 5). Similar anticoagulation properties of biosynthesized silver nanoparticles had also been previously reported [7, 41-44]. The formation of blood clot arising from infection can damage tissues and cause organ failure [45] and this may be linked with various cardiovascular disorders, autoimmune reactions, allergic responses, injuries, and emergence of cancerous cells [46,47]. The uncertainties and complications associated with conventional anticoagulants can be appropriately resolved and avoided via the applications of nanotechnology. Nanoparticles can be contrived to exclusively work together with the blood coagulation system to circumvent various blood disorders [48].
In order to avoid complications that may arise from the blood coagulation disorders, it is necessary to control the blood coagulation system to maintain a healthy state. This involves the prevention of aggregation of platelets, which can be achieved by AgNPs as previously demonstrated to inhibit the formation of thrombus [49]. The facts that AgNPs is non-toxic to platelets and its antimicrobial activities open a new way to treatment of thrombosis. Nanoparticles can as well impact varying degrees of influence on the blood coagulation system as a result of their size, charge, shape, and composition [48]. It can therefore, be inferred that the potency shown by AF-AgNPs obtained in this study can have useful applications in nanomedicine for the prevention of blood coagulation.

3.5 Thrombolytic activity of biosynthesized AgNPs

The AF-AgNPs dissolved the pre-formed blood clot after 90 min which revealed some amount of thrombolytic activity. The animal fur extract and the AgNO$_3$ solution used as the negative controls also yielded partial lyses of the blood clot. About 11, 13 and 25 % thrombolytic activities were observed for the precursor solution, animal fur extract and the biosynthesized AF-AgNPs (Figure 6). Also, the microscopic view of the lysed blood sample clearly showed red blood cells that were dispersed, an indication of the potency of the AF-AgNPs for application in nanomedicines. Similar observations had been reported of nanoparticles serving as anticoagulant and thrombolytic agents with potential for biomedical applications [7, 11, 12, 50].
3.6 Larvicidal activity of biosynthesized AgNPs

The AF-AgNPs displayed excellent larvicidal activities on mosquito larvae. Complete mortality (100%) of the tested mosquito larvae was achieved at 12 h for 60-100 µg/ml, 24 h for 20-40 µg/ml, and 36 h for 10 µg/ml (Figure 7). The results obtained indicate potency of the AF-AgNPs on the larvae, which is also comparable with reports of larvicidal activities of biosynthesized AgNPs against mosquito larvae [51-54].

![Figure 6. Thrombolytic activities (a, control; b, fur extract; c, AF-AgNPs)](image)

![Figure 7. Larvicidal activity of AF-AgNPs on Anopheles larvae](image)
3.7 Cytogenotoxic effects of AgNPs on Allium cepa

The cytotoxicity of the AgNO₃ solution, fur extract and AF-AgNPs at 24 h exposure revealed dose dependent reduction in dividing cells with mitotic index found less than half that of the control only at 100 µg/ml of the treatments (Table 1). Mitotic inhibition was also found to increase with increase in concentration across the concentrations. Numbers of dividing cell stages reflected dose dependent decrease with the lowest obtained at 100 µg/ml of the AF-AgNPs. Genotoxicity assessment revealed that all the three treatments showed potential to induce chromosomal aberrations with 100 µg/ml of AgNO₃ and fur extract inducing more aberrant cells than the AF-AgNPs. Trend in mitotic index and inhibition observed at 48 and 72 h were similar to that of 24 h, except that AF-AgNPs at 72 h showed more toxicity with only value obtained at 0.01 above half the value obtained for the control, suggesting the cytotoxicity of the AF-AgNPs (Tables 1-3). Observations similar to these were reported for A. cepa treated with different groups of pesticides [55-57]. However, unlike Cola pod mediated AgNPs [31], complete cell arrest was not observed in any of the equivalent concentrations used in the present study, indicating relatively low toxicity of the AF-AgNPs compared with those produced through plant sources. This probably suggests animal materials as better sources than plant materials for AgNPs synthesis due to reduced toxicity. The values of induced aberrations were higher in both AgNO₃ and fur extract at all concentrations compared to AF-AgNPs (Tables 2 and 3). Aberrant cells induced include stickiness, disturbed metaphase, c-mitosis, vagrant chromosomes and fragmentation (Figure 8). Exposure at all treatment concentrations revealed reduction in mitotic activities which could be due to the impairment of DNA synthesis [58, 59].

The growth inhibition revealed dose dependent decrease in mean root lengths with the value obtained for the control significantly higher compared to all concentrations in the three treatments (Table 4). The number of roots harvested also showed similar dose dependent decrease with highest percentage root inhibition observed at 100 µg/ml of each of the treatments. The observed results of growth inhibition corroborate that of cytogenotoxicity, which conform to the claim that before the occurrence of chromosome aberrations, some growth restrictions might have happened stemming from the cumulative response of all the damaging effects [60].
Figure 8. Various aberrations induced by the AF-AgNPs on *Allium cepa* (a, vagrant chromosome; b, chromosome bridge; c, c-mitosis; d, sticky chromosomes)

Table 1. Cytological effects of AF-AgNPs on *Allium cepa* after 24 h of treatment

| Conc. (ng/ml) | No of dividing cells | Mitotic index | Mitotic inhibition | Pro | Meta | Ana | Telo | SK | DA | DM | VC | FG | %aberrant cells | %aberrant per dividing cells | %aberrant per cells scored |
|--------------|----------------------|---------------|-------------------|-----|------|-----|------|----|----|----|----|----|----------------|-----------------------------|--------------------------|
| **AgNO₃ Solution** | | | | | | | | | | | | | | | | |
| Control     | 485                   | 9.70          | -                 | 151 | 115  | 116 | 103  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| 0.01        | 398                   | 7.9           | 17.94            | 106 | 97   | 101 | 94   | 2  | 2   | -  | -  | 4  | 1.00| 0.08 |
| 0.1         | 376                   | 7.52          | 22.47            | 100 | 92   | 93  | 91   | 1  | 1   | -  | -  | 3  | 0.79| 0.06 |
| 1           | 289                   | 5.78          | 40.41            | 84  | 70   | 72  | 63   | 2  | 2   | -  | -  | 4  | 1.38| 0.08 |
| 10          | 254                   | 5.08          | 47.62            | 76  | 63   | 58  | 57   | 2  | 1   | -  | -  | 4  | 1.57| 0.08 |
| 100         | 232                   | 4.64*         | 52.16            | 67  | 57   | 53  | 55   | 2  | 1   | -  | -  | 5  | 2.15| 0.10 |
| **Fur extract** | | | | | | | | | | | | | | | | |
| 0.01        | 470                   | 9.40          | 3.09             | 131 | 122  | 116 | 101  | 1  | -   | -  | -  | 1  | 0.21| 0.02 |
| 0.1         | 420                   | 8.40          | 13.40            | 113 | 107  | 103 | 97   | 1  | 1   | -  | -  | 2  | 0.47| 0.02 |
| 1           | 407                   | 8.14          | 16.08            | 106 | 99   | 101 | 101  | 2  | 1   | -  | -  | 3  | 0.73| 0.06 |
| 10          | 311                   | 6.22          | 35.87            | 93  | 76   | 75  | 67   | 3  | -   | -  | -  | 3  | 0.96| 0.06 |
| 100         | 149                   | 2.98*         | 69.27            | 48  | 37   | 35  | 29   | 5  | -   | -  | -  | 5  | 3.35| 0.10 |
| **AF-AgNPs** | | | | | | | | | | | | | | | | |
| 0.01        | 468                   | 9.36          | 3.50             | 146 | 112  | 104 | 106  | 1  | -   | -  | -  | 1  | 0.21| 0.02 |
| 0.1         | 415                   | 8.30          | 14.43            | 113 | 96   | 105 | 101  | 1  | -   | -  | -  | 1  | 0.24| 0.02 |
| 1           | 403                   | 8.06          | 16.90            | 121 | 106  | 91  | 85   | -  | 1   | -  | -  | 1  | 0.24| 0.02 |
| 10          | 305                   | 6.10          | 37.11            | 105 | 79   | 55  | 66   | -  | 1   | -  | -  | 1  | 0.33| 0.02 |
| 100         | 147                   | 2.94*         | 69.69            | 53  | 36   | 32  | 26   | 2  | -   | -  | -  | 2  | 1.36| 0.04 |

*Mitotic index less than half of the value of the control; SK, stickiness; DM, disturbed metaphase; DA, disturbed anaphase; VC, vagrant chromosomes; FG, fragmentation
Table 2. Cytological effects of AF-AgNPs on *Allium cepa* after 48 h of treatment

| Conc. (µg/ml) | No of dividing cells | Mitotic index | Mitotic inhibition | Pro | Meta | Ana | Telo | SK | DM | CM | VC | FG | CM | VC | FG | %aberrant cells | %aberrant per dividing cells | %aberrant per cells scored |
|---------------|----------------------|---------------|-------------------|-----|------|-----|------|----|----|----|----|----|----|----|----|-----------------|-----------------------------|-----------------------------|
| AgNO₃         |                      |               |                   |     |      |     |      |    |    |    |    |    |    |    |    |                 |                             |                             |
| Control       | 509                  | 10.18         | -                 | 163 | 122  | 108 | 116  | -  | -  | -  | -  | -  | -  | -  | -  | -               | -                           | -                           |
| 0.01          | 474                  | 9.48          | 6.87              | 148 | 110  | 109 | 107  | 1  | 1  | -  | -  | -  | -  | -  | -  | -               | 2                           | 0.42                        | 0.04                        |
| 0.1           | 449                  | 8.98          | 11.78             | 138 | 108  | 105 | 98   | 3  | -  | 1  | -  | 1  | 5  | 1  | 1  | 4               | 1.1                         | 0.10                        |
| 1             | 363                  | 7.26          | 28.68             | 97  | 89   | 91  | 86   | 1  | 1  | 2  | -  | -  | 4  | 1.10          | 0.08                       |
| 10            | 276                  | 5.52          | 45.77             | 78  | 68   | 66  | 64   | 2  | 2  | -  | 1  | 7  | 2.54          | 0.14                       |
| 100           | 229                  | 4.58          | 55.01             | 63  | 54   | 54  | 58   | 4  | 3  | 1  | -  | 8  | 3.49          | 0.16                       |
| Fur Extract   |                      |               |                   |     |      |     |      |    |    |    |    |    |    |    |    |    |                 |                             |                             |
| 0.01          | 496                  | 9.92          | 2.55              | 136 | 124  | 124 | 112  | 2  | -  | -  | -  | 2  | 0.40          | 0.02                       |
| 0.1           | 480                  | 9.60          | 5.69              | 129 | 121  | 121 | 120  | 107| 1  | 1  | -  | -  | 2  | 0.41          | 0.02                       |
| 1             | 433                  | 8.66          | 14.93             | 123 | 117  | 105 | 88   | -  | 2  | -  | -  | -  | 2  | 0.46          | 0.02                       |
| 10            | 271                  | 5.42          | 46.75             | 76  | 72   | 66  | 57   | 2  | -  | 1  | -  | 3  | 1.10          | 0.06                       |
| 100           | 131                  | 2.62          | 74.26             | 39  | 34   | 30  | 28   | 3  | -  | -  | 3  | 2.29          | 0.06                       |
| AF-AgNPs      |                      |               |                   |     |      |     |      |    |    |    |    |    |    |    |    |    |                 |                             |                             |
| 0.01          | 492                  | 9.84          | 3.33              | 157 | 115  | 117 | 103  | 1  | -  | -  | -  | 1  | 0.20          | 0.02                       |
| 0.1           | 474                  | 9.48          | 6.87              | 147 | 111  | 113 | 103  | 1  | -  | -  | -  | 1  | 0.21          | 0.02                       |
| 1             | 430                  | 8.60          | 16.67             | 131 | 105  | 104 | 90   | -  | 2  | -  | -  | 2  | 0.46          | 0.04                       |
| 10            | 268                  | 5.36          | 47.34             | 83  | 69   | 60  | 56   | -  | 2  | -  | -  | 2  | 0.75          | 0.04                       |
| 100           | 130                  | 2.60          | 74.45             | 41  | 34   | 28  | 27   | -  | -  | 2  | -  | 2  | 1.53          | 0.04                       |

*Mitotic index less than half of the value of the control; SK, stickiness; DM, disturbed metaphase; VC, vagrant chromosomes; FG, fragmentation; CM, c-mitosis
| Conc. (µg/ml) | Mitotic index | Mitotic inhibition | Pro | Meta | Ana | Telo | SK | DM | CM | VC | FG | % aberrant cells | % aberrant per dividing cells | % aberrant per cells scored |
|--------------|---------------|-------------------|-----|------|-----|------|----|----|----|----|----|----------------|-----------------------------|-----------------------------|
| AgNO₃        |               |                   |     |      |     |      |     |    |    |    |    |                |                             |                            |
| Control      | 490           | 9.80              | -   | 155  | 115 | 114  | 106| -  | -  | -  | -  | -  | -             | -                           | -                           |
| 0.01         | 392           | 7.84              | 20.00| 106  | 99  | 93   | 94 | 2  | 3  | 1  | -  | -  | 6             | 1.53                        | 0.12                        |
| 0.1          | 350           | 7.00              | 28.57| 99   | 90  | 83   | 78 | 2  | 4  | 2  | -  | 1  | 9             | 2.57                        | 0.18                        |
| 1            | 239           | 4.78              | 51.22| 67   | 56  | 54   | 62 | 5  | -  | 2  | -  | -  | 7             | 2.93                        | 0.14                        |
| 10           | 221           | 4.42*             | 54.89| 64   | 53  | 50   | 54 | 4  | 1  | 3  | -  | -  | 8             | 3.61                        | 0.16                        |
| 100          | 198           | 3.96*             | 59.59| 61   | 49  | 46   | 42 | 2  | 4  | 3  | -  | -  | 9             | 4.55                        | 0.18                        |
| Extract      |               |                   |     |      |     |      |     |    |    |    |    |    |                |                             |                            |
| 0.01         | 320           | 6.40              | 34.69| 88   | 82  | 77   | 73 | 3  | -  | -  | -  | -  | 3             | 0.93                        | 0.06                        |
| 0.1          | 278           | 5.56              | 43.26| 78   | 69  | 68   | 63 | 1  | 1  | 1  | -  | -  | 3             | 1.07                        | 0.06                        |
| 1            | 235           | 4.70              | 52.04| 72   | 58  | 55   | 50 | 3  | -  | 1  | -  | -  | 4             | 1.70                        | 0.08                        |
| 10           | 146           | 2.92*             | 70.20| 44   | 36  | 35   | 31 | 4  | -  | 1  | -  | -  | 5             | 3.04                        | 0.10                        |
| 100          | 104           | 2.04*             | 79.18| 30   | 26  | 25   | 23 | 6  | 1  | -  | -  | -  | 7             | 6.73                        | 0.14                        |
| AgNPs        |               |                   |     |      |     |      |     |    |    |    |    |    |                |                             |                            |
| 0.01         | 226           | 4.52              | 53.87| 74   | 59  | 51   | 42 | -  | 1  | -  | -  | -  | 1             | 0.44                        | 0.02                        |
| 0.1          | 205           | 4.10*             | 58.16| 68   | 54  | 46   | 37 | 2  | -  | -  | -  | -  | 2             | 0.97                        | 0.04                        |
| 1            | 186           | 3.72*             | 62.04| 64   | 49  | 41   | 32 | -  | 2  | -  | -  | -  | 2             | 1.07                        | 0.04                        |
| 10           | 141           | 2.82*             | 71.22| 53   | 38  | 30   | 20 | 2  | -  | -  | -  | -  | 2             | 1.42                        | 0.04                        |
| 100          | 102           | 2.04*             | 79.18| 42   | 28  | 20   | 12 | 2  | -  | 1  | 1  | -  | 4             | 3.92                        | 0.08                        |

*Mitotic index less than half of the value of the control; SK, stickiness; DM, disturbed metaphase; CM, c-mitosis; VC, vagrant chromosomes; FG, fragmentation
### Table 4. Toxic effects of silver nitrate salt on *Allium cepa* root growth

| Concentration (µg/ml) | AgNO$_3$ | Fur Extract only | AF-AgNPs |
|-----------------------|----------|------------------|----------|
|                       |          | Total (N)        | Mean root length±SE | %Mean root length | %Root inhibition | Total (N) | Mean root length±SE | %Mean root length | %Root inhibition | Control (N) | Mean root length±SE | %Mean root length | %Root inhibition |
|                       |          |                  |                      |                  |                 |           |                      |                  |                 |              |                      |                  |                 |
| Control               |          | 255              | 3.58±0.92            | 100              | -                | 255        | 3.58±0.92            | 100              | -                | 255          | 3.58±0.92            | 100              | -                |
| 0.01                  |          | 239              | 2.67±0.76            | 74.58            | 25.42            | 157        | 3.05±0.93            | 85.19            | 14.80            | 180          | 3.36±0.47            | 93.85            | 6.14             |
| 0.1                   |          | 242              | 2.43±0.61            | 67.87            | 32.12            | 150        | 2.69±0.81            | 75.13            | 24.86            | 153          | 2.55±0.47            | 71.22            | 28.77            |
| 1.0                   |          | 169              | 2.04±0.63            | 56.98            | 43.02            | 130        | 2.29±0.59            | 63.96            | 36.03            | 165          | 2.49±0.43            | 69.55            | 30.44            |
| 10.0                  |          | 213              | 1.39±0.43            | 38.83            | 61.17            | 123        | 1.02±0.38            | 28.49            | 71.50            | 112          | 0.47±0.31            | 13.12            | 86.87            |
| 100                   |          | 124              | 0.35±0.02            | 9.78             | 90.22            | 131        | 0.48±0.26            | 13.40            | 86.59            | 111          | 0.18±0.00            | 5.02             | 94.97            |

Mean with different subscript letter a, b, c, d and e are significantly different from each other (p< 0.05)
4 Conclusion

The present study has clearly demonstrated the usefulness of animal fur extract as a cost-effective and eco-friendly bio-resource in the green synthesis of silver nanoparticles (AF-AgNPs). The biosynthesized particles were spherical with size ranging from 11-32 nm. The highly remarkable larvicidal activities of AF-AgNPs against larvae of the vector of Plasmodium parasites (60-100 % after 12 h of exposure), including their anticoagulant and thrombolytic activities, indicate their relevance in biomedical applications. Furthermore, the remarkable antioxidant activities (hydrogen peroxide and DPPH scavenging) suggest the biotechnological potential of the synthesized nanoparticles in the biomedical industry and treatment of industrial and domestic waste water. The success recorded in the use of animal fur, which in most instances are burnt or left to constitute nuisance in the environment, in the synthesis of AgNPs provides opportunity to turn waste to wealth. However, the synthesized AF-AgNPs exhibited some levels of cytogenotoxicity, which calls for caution in its application.

References

1. Aitken RJ, Chaudhry MQ, Boxall ABA and Hull M 2006 Manufacture and use of nanomaterials: current status in the UK and global trends. Occup. Med. 56 pp 300-306.
2. Benelli G 2016 Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res. 115 pp 23-34.
3. Boutonnet M, Lögdberg S, and Svensson EE 2008 Recent developments in the application of nanoparticles prepared from w/o microemulsions in heterogeneous catalysis. Curr. Opin. Colloid Interf. Sci 13 pp 270-286.
4. Koçak A and Karasu B 2018 General Evaluations of nanoparticles. El-Cezerî J. Sci. Eng. 5 (1) pp 191-236.
5. Oladipo IC, Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Akinwale AS, Gueguim-Kana EB and Beukes LS 2017 Green synthesis and antimicrobial activities of silver nanoparticles using cell-free extracts of Enterococcus species. Not. Sci. Biol. 9 (2): 196-203.
6. Oladipo IC, Lateef A, Elegbede JA, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Gueguim-Kana EB, Beukes LS, Oluyide TO and Atanda OR 2017 Enterococcus species
for the one-pot biofabrication of gold nanoparticles: characterization and nanobiotechnological applications. *J. Photochem. Photobiol. B: Biol.* **173** pp 250-257.

7. 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.

8. 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.

9. 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. Progress.* **35** e2829. [https://doi.org/10.1002/btpr.2829](https://doi.org/10.1002/btpr.2829).

10. Rao KJ and Paria S 2015 *Aegle marmelos* leaf extract and plant surfactants mediated green synthesis of Au and Ag nanoparticles by optimizing process parameters using Taguchi method. *ACS Sustain. Chem. Eng.* **3** (3) pp 483-491.

11. 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 433-442.

12. 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.

13. Ismail M, Gul S, Khan MI, Khan MA, Asiri AM Khan SB 2019 Green synthesis of zerovalent copper nanoparticles for efficient reduction of toxic azo dyes congo red and methyl orange. *Green Process Synth.* **8** pp 135-143.
14. Lateef A, Ojo SA, 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 (6) pp 863-874.

15. 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.* 6 140. [http://dx.doi.org/10.1007/s13205-016-0459-x](http://dx.doi.org/10.1007/s13205-016-0459-x).

16. Khatami M, Iravani S, Varma RS, Mosazade F, Darroudi M and Borhani F 2019 Cockroach wings-promoted safe and greener synthesis of silver nanoparticles and their insecticidal activity. *Bioprocess Biosys. Eng.* 42 (12) pp 2007-2014.

17. Balasooriya ER, Jayasinghe CD, Jayawardena UA, Ruwanthika RW, Mendis de Silva R and Udagama PV 2017 Honey mediated green synthesis of nanoparticles: new era of safe nanotechnology. *Journal of Nanomaterials* Article ID 5919836. [https://doi.org/10.1155/2017/5919836](https://doi.org/10.1155/2017/5919836).

18. Lee KJ, Park SH, Govarthanan M, Hwang PH, Seo YS, Cho M, Lee WH, Lee JY, Kamala-Kannan S and Oh BT 2013 Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens. *Mater Lett.* 105 pp 128-131.

19. Velmurugan P, Shim J, Kamala-Kannan S, Lee KJ, Oh BT, Balachandar V and Oh BT 2011 Crystallization of silver through reduction process using *Elaeis guineensis* biosolid extract. *Biotechnol. Progress* 27 (1) pp 273-279.

20. Tsyzdel M, Sztajnowski S, Michalak M, Wrzosek H, Kowalska S, Krucińska I and Lipp-Symonowicz B 2009 Structure and physical and chemical properties of fibres from the fifth larval instar of caddis-flies of the species *Hydropsyche angustipennis*. *Fibres Text. East. Eur.* 6 (77) pp 7-12.

21. 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 (1) pp 29-35.

22. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT and Yacaman MJ 2005 The bactericidal effect of silver nanoparticles. *Nanotechnol.* 16 (10) 2346. [https://doi.org/10.1088/0957-4484/16/10/059](https://doi.org/10.1088/0957-4484/16/10/059).
23. Williams BW, Cuverlier, ME, Berset C 1995. Use of free radical method to evaluate antioxidant activity. *Food Sci. Technol. LWT* 28 pp 25-30.

24. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR 2008 Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacol. Online.* 2 (9) pp 560-567.

25. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M and Eslami B 2009 *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacogn. Mag.* 5 (18) pp 122-126.

26. Sousa A, Ferreira IC, Barros L, Bento A and Pereira JA 2008 Effect of solvent and extraction temperatures on the antioxidant potential of traditional stoned table olives “alcaparras”. *LWT-Food Sci. Technol.* 41 (4) pp 739-45.

27. Cicha I 2015 Thrombosis, novel nanomedical concepts of diagnosis and treatment. *World J. Cardiol.* 7 (8) pp 434-441.

28. Bansal SK, Singh KV and Kumar S 2009 Larvicidal activity of the extracts from different parts of the plant *Solanum xanthocarpum* against important mosquito vectors in the arid region. *J. Environ. Biol.* 30 (2) pp 221-226.

29. Bakare AA, Mosuro AA and Osibanjo O 2000 Effects of simulated leachates on chromosomes and mitosis of *Allium cepa* (L). *J. Environ. Biol.* 21 pp 263-271.

30. Yekeen TA, Ayandele AA, Akinboro A, Onipede AY and Alawode R 2013 Assessment of toxicity and microbial attributes of synthetic epoxy resin and acetaminophen effluents. *Pollut. Res.* 32 (4) pp 9-16.

31. 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 extract-mediated silver nanoparticles (AgNPs) using an *Allium cepa* assay. *J. Taibah Univ. Sci.* 11 (6) pp 895-909.

32. 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.

33. Jeevan P, Ramya K and Rena AE 2012 Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. *Indian J. Biotechnol.* 11 (1) pp 72-76.
34. Zakir S, El-Kady MF, and Abd-El-Haleem D 2011 Biosynthesis and structural characterization of silver nanoparticles from bacterial isolates. *Mater. Res. Bull.* **46** (2) pp 1571-1576.

35. Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H and Sangiliyandi G 2008 Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. *Mater. Lett.* **62** (29) pp 4411-413.

36. Shankar S, Jaiswal L, Aparna RSL and Prasad RGSV 2014 Synthesis, characterization, *in vitro* biocompatibility, and antimicrobial activity of gold, silver and gold silver alloy nanoparticles prepared from *Lansium domesticum* fruit peel extract. *Mater. Lett.* **137**: 75-78.

37. Kannan RR, Arumugam R, Ramya D, Manivannan K and Anantharaman P 2013 Green synthesis of silver nanoparticles using marine macroalga *Chaetomorpha linum*. *Appl. Nanosci.* **3** (1) pp 229-233.

38. Mehmood A, Murtaza G, Bhatti TM, Raffi M and Kausar R 2014 Antibacterial efficacy of silver nanoparticles synthesized by a green method using bark extract of *Melia azedarach* L. *J. Pharmaceut. Innov.* **9** (3) pp 238-245.

39. Shanmugam C, Sivasubramanian G, Parthasarathi B, Baskaran K, Balachander R and Parameswaran V 2016 Antimicrobial, free radical scavenging activities and catalytic oxidation of benzyl alcohol by nano-silver synthesized from the leaf extract of *Aristolochia indica* L.: a promenade towards sustainability. *Appl. Nanosci.* **6** (5) pp 711-723.

40. Elegbede JA and Lateef A 2019 Green synthesis of silver (Ag), gold (Au) and silver-gold (Ag-Au) alloy nanoparticles: A review on recent advances, trends and biomedical applications. In: Verma DK, Goyal MR and Suleria HAR (Eds.). *Nanotechnology and Nanomaterial Applications in Food, Health and Biomedical Sciences*. https://doi.org/10.1201/9780429425660-1. Apple Academic Press Inc. /CRC Press, Taylor and Francis Group, Oakville, Ontario, Canada. ISBN 978-1-77188-764-9. Pp. 3-89.

41. Jeyaraj M, Varadan S, Anthony KJ, Murugan M, Raja A and Gurunathan S 2013 Antimicrobial and anticoagulation activity of silver nanoparticles synthesized from the culture supernatant of *Pseudomonas aeruginosa*. *J. Ind. Eng. Chem.* **19** (4) pp 1299-1303.
42. 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. Ind. Eng. Chem.* 29 pp 257-264.

43. 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 (1) pp 149-164.

44. 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.

45. Levi M, Schultz M and van der Poll T 2010 Disseminated intravascular coagulation in infectious disease. *Sem. Thromb. Hemost.* 36 (4) pp 367-377.

46. Davalos D and Akassoglou K 2012 Fibrinogen as a key regulator of inflammation in disease. *Sem. Immunopathol.* 34 (1) pp 43-62.

47. Prandoni P, Falanga A and Piccioli A 2007 Cancer, thrombosis and heparin-induced thrombocytopenia. *Thromb. Res.* 120 pp S137-S140.

48. Ilinskaya AN and Dobrovolskaia MA 2013 Nanoparticles and the blood coagulation system. Part II: safety concerns. *Nanomed.* 8 (6) pp 969-981.

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

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

51. Gnanadesigan M, Anand M, Ravikumar S, Maruthupandy M, Vijayakumar V, Selvam S, Dhineshkumar M and Kumaraguru AK 2011 Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. *Asian Pacif. J. Trop. Med.* 4 (10) pp 799-803.

52. Priyadarshini KA, Murugan K, Panneerselvam C, Ponarulselvam S, Hwang JS and Nicoletti M 2012 Biolarvicidal and pupicidal potential of silver nanoparticles synthesized
using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol. Res.* **111** (3) pp 997-1006.

53. 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** (10) pp 1295-1306.

54. 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.

55. Asita AO and Matebesi LP 2010 Genotoxicity of hormoban and sevenother pesticides to onion root tip meristematic cells. *Afr. J. Biotechnol.* **9** (27) pp 4225-4232.

56. Shaikh S, Nazam N, Lone MI and Ahmad W 2012 Dichlorophen and Dichlorovos mediated genotoxic and cytotoxic assessment on root meristem cells of *Allium cepa*. *Sci. Diliman.* **24** (1) pp 13-22.

57. Yekeen TA and Adeboye MK 2013 Cytogenotoxic effects of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan pesticides on *Allium cepa* root cells. *Afr. J. Biotechnol.* **12** (41) pp 6000-6006.

58. Schneiderman MH, Dewey WC and Highfield DP 1971 Inhibition of DNA synthesis in synchronized Chinese hamster cells treated in G1 with cycloheximide. *Exp. Cell Res.* **67** (1) pp 147-155.

59. Sudhakar R, Gowda KNN and Venu G 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytol.* **66** pp 235-239.

60. Fiskesjo G 1997 *Allium* test for screening chemical evaluation of cytological parameters. In Wang W, Gorsuch JW and Hughes JS (Eds), Plants for Environmental Studies, New York, NY: CRC Lewis Publishers, pp 307-333.