Nano Plus: Sci. Tech. Nanomat. 2 (2021) 136 – 154
https://doi.org/10.48187/stnanomat.2021.2.008

Nano Plus: Science and Technology of Nanomaterials
Journal home page: https://www.stnanojournal.org/
ISSN: 2782-8166 (print) 2782-8174 (electronic)

Biological Activities and Cytogenotoxicity Evaluation of Green Synthesized Corn Silk-Mediated Silver Nanoparticle

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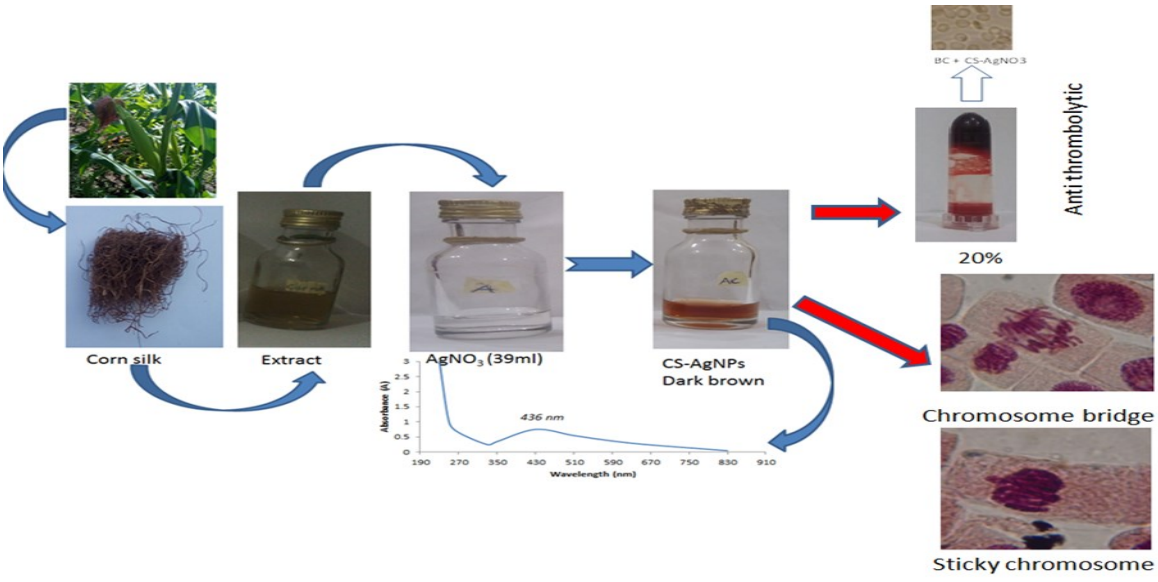
Highlights

- Corn silk extract was able to mediate the synthesis of silver nanoparticles CS-AgNPs
- Synthesized CS-AgNPs possessed larvicidal, antioxidant, and ant thrombolytic properties
- The toxicity potential of the treatments was in the order of $AgNO_3$ < CS-AgNPs< CS-extract
- Mitodepressant potential of CS-AgNPs can be explored in the anticancer therapy

Graphical Abstract



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https://doi.org/10.48187/stnanomat.2021.2.008

Article info

Received: October 1, 2021
Revised: October 14, 2021
Accepted: October 16, 2021

Keywords:
Nanoparticles,
Silver,
Chromosome,
Aberration,
Allium cepa,
Corn silk

Abstract

Nanoparticles have been proven to have a wide range of applications due to their peculiar properties. Of the metallic nanoparticles, green synthesized silver nanoparticles (AgNPs) have been widely used in various applications. This study evaluated the potentials of corn silk extract in the synthesis of silver nanoparticles (CS-AgNPs), its biological activities as well as the safety. Powdered corn silk (1g) was warmed in 100 ml of distilled water at 60°C for 1h, cooled, and centrifuged at 4000 rpm for 30 minutes. Then, 1ml of the extract was added to 40 ml of 1mM $AgNO_3$ to reduce Ag^+ to nano size. Characterization of CS-AgNPs was by UV-visible spectroscopy, Fourier-transform-infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and Energy Dispersive X-ray (EDX) analysis. The efficacy of CS-AgNPs as a larvicidal, anticoagulant, thrombolytic, and antioxidant agent was evaluated. Safety evaluation was carried out using *Allium cepa* assay where 0.01, 0.1, 1.0, 10.0, and 100.0 µg/ml of the CS-AgNPs, and Ag-salt, as well as CS-extract were used. Twenty (20) onions were exposed per concentration while distilled water was used as diluent and control. Data were analyzed through Analysis of variance and Duncan tests. The CS-AgNPs was dark brown with surface Plasmon resonance peak at 436 nm. FTIR revealed the availability of biomolecules responsible for capping and as stabilizing agents. TEM revealed nanoparticles of size range 8.48-45.78 nm. The CS-AgNPs caused 100% mortality of exposed *Anopheles* mosquito larvae in 12 h, and showed a good anticoagulant and thrombolytic potential on human blood for the prevention of blood clots. Antioxidant activity using DPPH and H_2O_2 scavenging assays revealed the potentials of CS-AgNPs in absorbing and neutralizing free radicals. The synthesized CS-AgNPs and Ag-salt solution induced more chromosomal aberrations at 24 and 48 h and only Ag-salt solution induced more aberrant cells at 72 h. Root growth inhibition observed correlate with the observed mitotic inhibition at higher concentrations especially for CS-AgNPs. Mitodepressant potentials especially at higher concentrations can be explored as an anticancer agent along with other established potentials.

1. Introduction

Nanotechnology is an emerging field of study which involves the synthesis and characterization of metallic nanoparticles such as silver, gold, platinum, and metal oxides such as ZnO, CuO, and TiO_2 for various applications. Nanoparticles are in the range of 1-100 nm and reported to show different properties which includes chemical, optical, mechanical, electronic, and magnetic better than larger particles due to possession of specific characteristics such as shape, size, and atomic distribution [1]. Many methods are employed for the synthesis of nanomaterials such as heat evaporation [2], chemical reduction [3], photochemical [4], electrochemical [5], reverse micelle [6], thermal decomposition [7], radiation [8] and microwave-assisted [9] methods. Most of these methods require the use of hazardous chemicals and high energy for the preparation of nanoparticles. Trending in the synthesis of nanoparticles is the use of biological methods due to their cost effectiveness, eco-friendliness, and being less toxic compared to both physical and chemical methods [10]. Biological methods have also been proven to be better methods due to slower kinetics, ease of manipulation and control over crystal growth and their stabilization [11].

The applications of nanotechnology include optical sensors, drug delivery, bioengineering, cosmetics, biological labeling, biotechnology, catalysis, water treatment, and the detection of genetic disorders [1, 12]. Its uses have been extended to textiles, keyboards, wound dressings, and biomedical devices [13]. Silver nanoparticles have been successfully synthesized from agro-industrial wastes [14,15] as well as animal waste and their

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metabolites [16, 17, 18], with their extracts serving as reducing, capping, and stabilizing agents. Synthesized AgNPs and other nanoparticles are known to have diverse activities ranging from antimicrobial, antioxidant, larvicidal to catalytic. Reports had also shown the potentials of nanoparticles to be cytotoxic against cancer cells [19]. The use of the agro-wastes, which are often discharged into the environment with the attendant pollution, in the synthesis is assisting in the conversion of waste to wealth.

The biosynthesis of nanoparticles involves the use of natural materials such as microorganisms [20, 21, 22], plants [23, 24], and animal materials [1, 16, 17, 18]. Plants are known to contain various secondary metabolites such as alkaloids, terpenoids, flavonoids, and tannins which serves as suitable reducing agent for nanoparticle synthesis and also provides stabilization. Biopolymers such as cellulose, chitosan, alginate, dextran, and tree gums are another family of natural sources biomolecules which are used for the reduction and stabilization of nanoparticles [19, 25]. The use of plants waste products for the synthesis of nanoparticles emerged as an interesting methodology for the preparation of biologically active nanoparticles with minimal cost, rapid and dependable process, with little or no adverse effects on human beings and the environment.

Corn silk is the long, thread-like strands that grow underneath the husk of a fresh cob of maize plant. It is used in traditional herbal medicine practices to produce beverages for diet and to induce urination. Also, it is used as a soothing, anti-inflammatory diuretic that directly reduces painful symptoms and swelling due to inflammation [26]. The constituents of corn silk include proteins, vitamins, carbohydrates, calcium, potassium, magnesium and sodium salt, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids [27. 28]. In most cases, corn silk is mainly treated as waste.

Currently, with increased concerns for environmental pollution, the *Allium cepa* test has emerged as a reliable tool for the prediction of the environmental impact of disposed drugs, herbicides as well as the engineered nanomaterials at the end of their life cycle [29] or in toxicity studies before their introduction into the environment [18, 30, 31]. There are lots of research works in toxicity and other environmental studies where *Allium cepa* assay had been used which further corroborates its efficacy [32, 33, 34, 35].

Due to the environmental toxicity of nanoparticles from physical and chemical processes, our goal in our laboratory is to source various plant materials as well as animal materials for the synthesis. We also dwell on various applications of biogenic nanoparticles, explore their usefulness and evaluate their potential in biological activities as well as laying emphasis on their biosafety via cytotogenotoxicity studies and other assays. Therefore, this study was developed to evaluate the potential of corn silk extract in the synthesis of silver nanoparticles, explore the biological activities as well as its safety through cytogenotoxicity assay.

2. Materials and Methods

2.1 Procurement of materials

Corn silk used in this study was obtained from LAUTECH farm. The variety used was solo corn (*Zea mays* L. family Poaceae). It was identified further at the herbarium of the Department of Pure and Applied Biology. Silver nitrate ($AgNO_3$) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich agent, Nigeria, while all other chemical materials were of the analytical grade

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2.2 Preparation of corn silk extract

Samples of corn silks (*Zea mays* L. Poaceae) obtained in fresh form were washed thoroughly in the laboratory to remove dust, air-dried, and then ground into powder. The corn silk extract was made by adopting the modified method of Tszydel *et al.* [36]. A 1g of corn silks powder was added to 100 ml of distilled water and heated at 60 °C for 1h. The extract was allowed to cool to room temperature (25 °C±1), filtered using Whatman's filter paper 42, and then centrifuged at 4000 rpm for 30 minutes. The supernatant obtained was designated as CS-extract and store at 4°C for further use.

2.3 Green synthesis of CS-AgNPs and characterization

The CS-extract was used to synthesis silver nanoparticles (AgNPs) as described by Lateef *et al.* [20]. A 1ml of the extract was added to the reaction vessel containing 40 ml of 1 mM silver nitrate ($AgNO_3$) solution for the reduction of Ag^+ to its nano size Ag^o. The reaction was photoactivated under sunlight for 15 minutes. The formation of CS-AgNPs was monitored visually for a colour change. UV-visible spectrophotometer operated at 200-900 nm was then used for further confirmation of the synthesis. Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), and energy-dispersive X-ray (EDX) analyses were respectively used for the determination of biomolecules involved in the green synthesis, evaluation of particles size and their morphology, and elemental composition of the CS-AgNPs [17, 23].

2.4 Larvicidal study for the CS-AgNPs efficacy

The larvae of Anopheles mosquito used were collected into a large covered container from stagnant water and were used for the experiment. The efficacy of the CS-AgNPs against Anopheles mosquito larvae was assayed using the WHO [37] recommended guideline. A 10 ml of CS-AgNPs (10, 20, 40, 60, 80, and 100 µg/ml) was used for the toxicity test against 10 *Anopheles* mosquito larvae, while distilled water was used as control. The test was carried out in triplicate and readings were taken at 6 h intervals [18]. The percentage mortality was plotted against the time of exposure to the CS-AgNPs.

2.5 Anticoagulant activity for CS-AgNPs

The anticoagulant activity was performed by mixing 0.5 ml of 150 µg/ml of CS-AgNPs with 5 ml of freshly collected human blood from an adult volunteer. Also set up was a control experiment in which 0.5 ml of blood was introduced into EDTA bottle. The reaction mixtures were held for 1 h at room temperature (25±1°C) after which it was observed under the microscope [16, 17].

2.6 Thrombolytic activity

The CS-AgNPs were evaluated for thrombolytic activity using human blood as previously described [18, 38]. For this assay, 5 ml of venous blood collected from healthy volunteers was dispersed in sterile pre-weighed microcentrifuge tubes, followed by incubation at 37 °C for 45 minutes. The blood clot was allowed to form, then serum was removed carefully and tubes were weighed. The CS-AgNPs (100 µg/ml) was added to the blood clot formed and then incubated at room temperature of 37 °C for 90 minutes. The developed fluid from the clot was removed and tubes were again weighed to measure the lysed clot. The Ag-salt and corn silk extract (100µl) were used as positive controls and distilled water was used as the negative control. Percentage of clot lysed was

$$\% \text{ Thrombolysis} = \frac{\text{weight of clot after treatment}}{\text{weight of clot before treatment}} \times 100$$

expressed as:

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2.7 Antioxidant activities

2.7.1 Free radical scavenging activity using DPPH

The ability of the CS-AgNPs to mop up free radicals was evaluated using the methanolic solution of 0.1 mM DPPH . DPPH (3 ml) was introduced into each of the 6 bijou bottles. Thereafter, 1 ml of each graded concentration (1, 2, 5, 10, 20, and 40 µg/ml) of CS-AgNPs was added to the prepared DPPH solution. The solutions were shaken and incubated in a dark box at room temperature for 30 minutes. The absorbance was then read at 517 nm against absolute methanol as blank [18, 20, 39], while ascorbic acid was used as a standard control. The antioxidant activity of the biosynthesized CS-AgNPs was calculated as follows;

$$\%\text{Inhibition} = \frac{\text{A blank} - \text{A sample}}{A\ \text{blank}} \times 100$$

2.7.2 Hydrogen peroxide free radical scavenging activity

The free radical scavenging activity of the CS-AgNPs was determined using hydrogen peroxide [18, 40]. A solution of H_2O_2 (40 mM) was prepared in a phosphate buffer of pH 7.4. Four (4) ml each of 1, 2, 5, 10, 20, 40, 60, and 80 µg/ml of CS-AgNPs was prepared and 0.6 ml, 40 mM of H_2O_2 solution was added. The absorbance was read at 610 nm after 20 minutes against a blank solution (phosphate buffer with H_2O_2).

$$\%\ \text{scavenging effect} = \frac{A0 - A1}{A1} \times 100$$

Where, A_0 is the absorbance of the control and A1 is the absorbance of the test sample. Inhibition percentage was plotted against concentrations.

2.9 Cytotogenotoxicity evaluation using *Allium cepa* assay

Onion bulbs (250) of approximately equal size were purchased at Waso market in Ogbomoso and sun-dried for two weeks. The outer scales and brownish bottom plates of the onions were carefully removed, without tampering with the root ring primordial. Five different concentrations (0.01, 0.1, 1.0,10.0, and 100.0 µg/ml) each of CS-AgNPs and Ag-salt were prepared from the stock and twenty (20) onion bulbs exposed per concentration in pre-filled beakers(50 ml). Distilled water was used as diluents and control. Onion roots were grown at room temperature (25±1°C) in a dark cupboard with freshly prepared concentrations used at every 24 h. For microscopic evaluation, root tips were harvested from five *Allium cepa* per concentration at 24, 48, and 72 h, then fixed in ethanol-ethanoic acid (3:1 v/v). The root tips were then hydrolyzed in 1 N HCl at 65°C for 3 minutes. Two root tips, after maceration on slides, were stained with aceto-carmine for 15 minutes. One thousand (1,000) cells were scored per slide and a total of 5000 cells were scored per concentration for occurrences and frequency of different types of chromosomal aberrations [33, 41, 42, 43]. The photo-micrographs were taken with the Ocular VGA adapted Bresser Erudit DLX microscope (Germany). The mitotic index, mitotic inhibition, and percentage of chromosomal aberration for dividing cells were calculated based on these formulae:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells scores}} \times 100$$

$$\text{Mitotic inhibition} = \frac{(\text{Mitotic index of control} - \text{Mitotic index of treated})}{\text{Mitotic index of control}} \times 100$$

$$\%\ \text{of observed aberration} = \frac{\text{number of aberrant cells}}{\text{total number of cells counted}} \times 100$$

For microscopic evaluation at 72 h, roots from 5 *Allium cepa* per concentration were measured, and growth inhibition was evaluated. The EC_{50} values were obtained

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from the growth curves of percentage root length relative to control against various concentrations of the green synthesized CS-AgNPs and Ag-salt.

2.10 Statistical analysis

The root lengths of the treated bulbs at different concentrations and control were compared using one-way analysis of variance, while Duncan multiple range test was used as mean separator at 0.05 level of significance using SPSS 20.0. The Prism 6 statistical software was used for plotting of the graphs and EC_{50} calculation.

3. Results and Discussion

3. 1. Biosynthesis and characterization of AgNPs

The extract of corn silk mediated the production of CS-AgNPs, with the development of a dark-brown colour which stabilized within 15 minutes (Figure. 1), thus indicates the formation of CS-AgNPs. Lateef *et al.* [45] using *Chasmanthera dependens* and *Petiveria alliacea* plant materials, respectively, while reddish brown colouration was reported for corn silk AgNPs [28]. Lateef *et al*. [20] reported the formation of dark brown AgNPs solution using the crude extracellular keratinase of the strain of *B. safensis* LAU 13, Jeevan *et al*. [46] reported the formation of yellowish-brown AgNPs using cultured supernatant of *Pseudomonas aeruginosa,* while Oladipo *et al*. [22] reported dark brown AgNPs using cell free-extracts of *Enterococcus* species. The variations in colour may be attributed to components of the extract used for the synthesis of silver nanoparticles. Plant polyphenols, flavonoids, and reducing sugars were reported to be the main substances in many plants that exert reducing power [28, 47]

The Uv-vis spectrum of synthesized CS-AgNPs had a broad absorbance peak at the wavelengths of 436 nm (Fig. 1), which

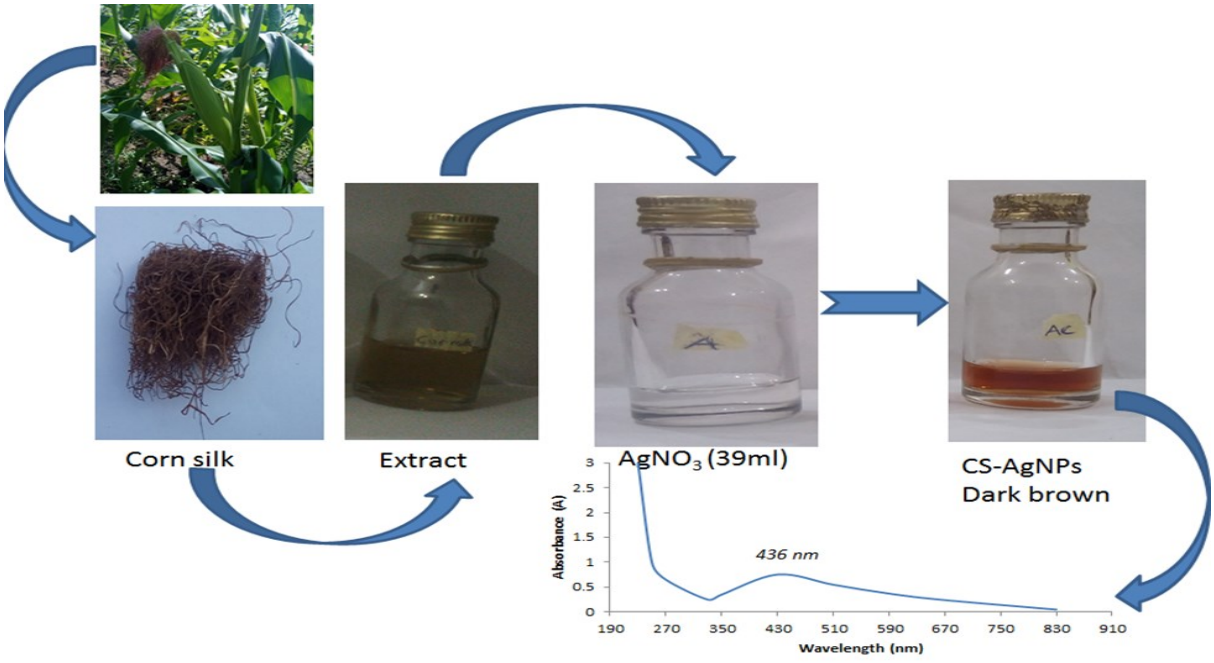

Fig. 1: Biosynthesized corn silk mediated AgNPs with UV-vis peak at 436 nm

Several authors have reported different colours for AgNPs solution which includes dark brown reported by Aina *et al.* [44] and conforms within the reported range of 400 – 450 nm for AgNPs [16. 17, 48]. The FTIR analysis of the synthesized CS-AgNPs

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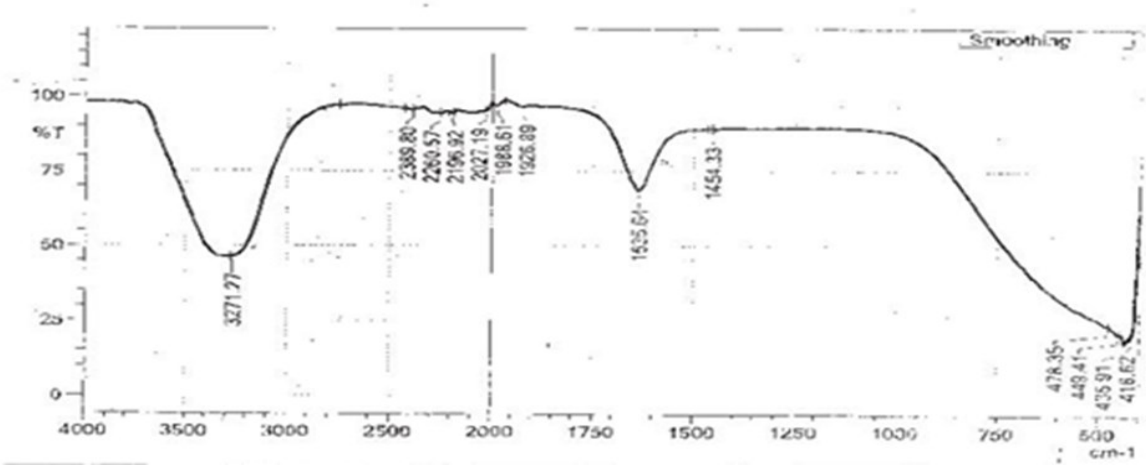

Fig. 2: FTIR analysis of AgNPs synthesized using corn silk extract

(Figure. 2) showed absorption peaks at 3271 and 1635 cm^{-1} corresponded to N−H of amines or O−H stretch of carboxylic acid and C=C stretch of alkenes or C=O stretch of amides, respectively. The peaks observed were similar to previously reported peaks [28, 49]. Our report indicates proteins as the major biomolecule acting as capping and stabilizing agents similar to earlier reports [28,50].

The size and morphological structure of synthesized AgNPs as shown by TEM revealed that the CS-AgNPs were spherical with sizes ranging from 8.49 – 45.78 nm (Fig. 3a). The observed lower range of nanoparticle size in this study was similar to the range reported for corn silk mediated AgNPs [28] and Corn silk mediated ZnO-NPs [51]. This shows the good stability of CS -AgNPs synthesized against agglomeration. The selected electron area diffraction (SAED) patterns showed the characteristics of the single and poly-crystalline configuration of AgNPs (Fig. 3b) in a ring-like manner as previously reported [17, 20]. EDX pattern (Fig. 3c) revealed the predominant presence of silver in the AgNPs solution. A similar observation was reported by Salem *et al.* [52] for AgNPs synthesized from latex and leaf extract of *Ficus sycomorus*. The elements contained in the AgNPs as revealed by the EDX analysis included carbon, oxygen, potassium, sodium, sulphur, silicon (Si), chlorine, and Ag. The Si peak was attributed to the Si substrate [53]. Since AgNPs were synthesized using corn silk extract (biomaterials), the presence of carbon and oxygen elements was inevitable. This study thus revealed that corn silk which is often considered an agricultural wastes

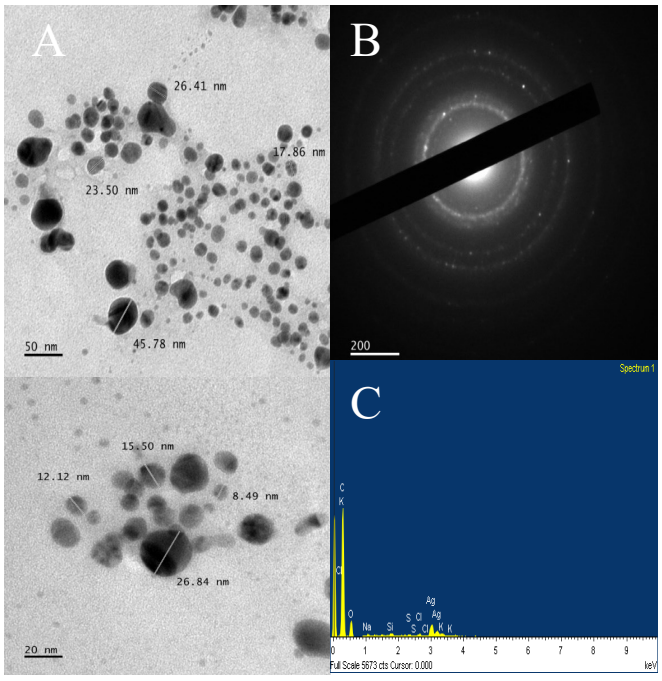

Fig. 3: The TEM micrographs (A), SAED patterns (B) and EDX spectra (C) of the biosynthesized corn silk AgNPs

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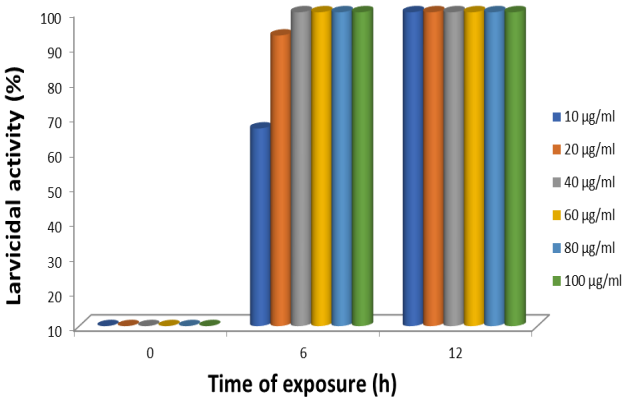

Fig. 4: Larvicidal activity of CS-AgNPs on Anopheles larvae

material could be used as a novel biomaterial for the green and eco-friendly synthesis of AgNPs.

3.2 Larvicidal activity of synthesized CS-AgNPs

The CS-AgNPs displayed high-level efficacy of toxicity to the Anopheles mosquito larvae, with complete mortality (100%) recorded during 6 – 12h of exposure. At 6 h, 100% mortality was recorded for 40-100 µg/ml. At 12h, 100% mortality was achieved at all tested concentrations of AgNPs 10-100µg/ml (Fig. 4). In the control, there was no record of mortality of larvae. The results obtained indicate larvicidal potency of the CS-AgNPs to the Anopheles mosquito larvae. Similar observations were reported by Roopan *et al.* [54] and Velux *et al.* [55] using *Cocos nucifera* coir extract and *Arachis hypogaea* peel extracts mediated silver nanoparticles. The larvicidal activity of CS-AgNPs to the mosquito larvae was however more potent compared to animal (goat) fur mediated AgNPs under the same concentrations and environmental conditions where complete mortality took 36 h [18].

3.3 Anticoagulant activity of CS-AgNPs

The CS-AgNPs prevented coagulation of fresh human blood similar to what was obtained in EDTA bottle as evidenced under the microscope, which revealed typical disc-shaped red blood cells (Fig. 5). In the control experiment, the blood clot was formed in fresh blood collected in an ordinary clean bottle. The examination of the nanoparticles treated blood under microscope showed the preservation of the structure of red blood cells. This outcome conformed with the reports on the anticoagulant properties of biogenic AgNPs [21, 56, 57]. The anti-

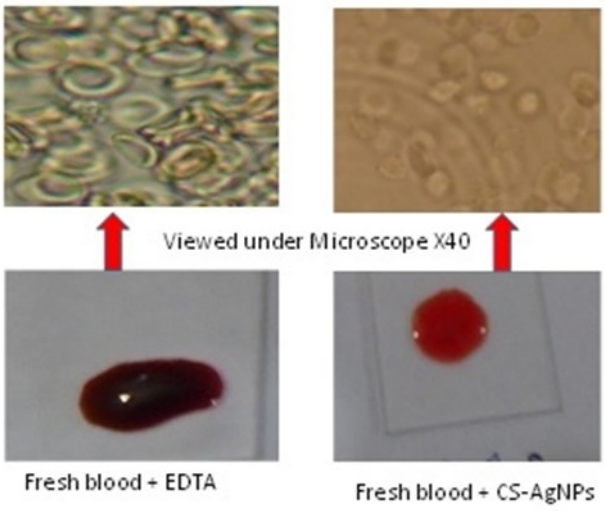

Fig. 5: Anticoagulant activity of the green synthesized CS-AgNPs

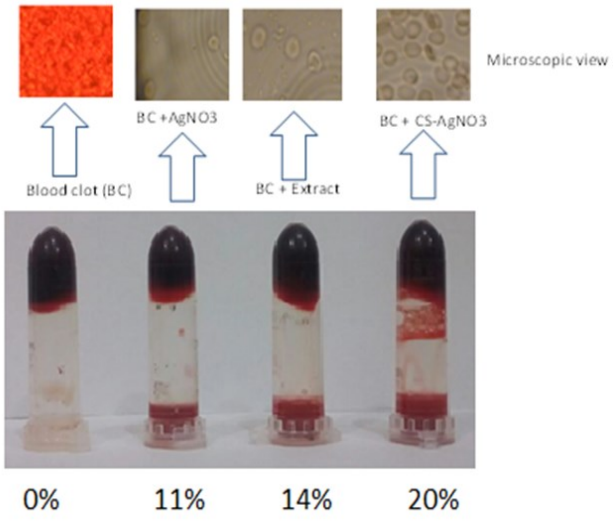

Fig. 6: Thrombolytic activities of the biosynthesized CS-AgNPs

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coagulation characteristic as observed could be very valuable in nanomedicine to avoid coagulation of blood. This also implies that such nanoparticles can be used as a drug carrier or as coating agents on medical instruments [58]. The coagulation of blood can lead to serious problems such as strokes, heart attacks, deep vein thrombosis, and pulmonary embolism. The anticoagulant potential of CS-AgNPs can be explored in nanomedicine for the prevention of blood coagulation.

3.4 The thrombolytic activity of CS-AgNPs

The CS-AgNPs showed high degrees of thrombolytic activity (20%) compared to CS-extract (14%)by rapidly dissolved pre-formed human blood clots within 15 minutes (Figure. 6). This is because CS-extract and CS-AgNPs prevented the aggregation of platelets. The Ag salt solution used as control also revealed partial lysis of the blood clot (11%), while the negative control displayed no thrombolytic activity (0%). The microscopic view of the lysed blood showed the dispersal of cells due to the reaction of thrombus with CS-AgNPs. The formation of a thrombus in the blood circulatory system can cause considerable damage through blockage of the cardiovascular system and ultimately death [59]. Therefore, CS-AgNPs potential to efficiently dissolve the blood clots is an indication of its potency for suspension of thrombus and possible application in nano-medicines. Similar observations were previously reported for different forms of green synthesized nanoparticles [14, 18, 60].

3.5 Antioxidant activity of CS-AgNPs

3.5.1 DPPH – free radical scavenging

The DPPH radical scavenging activity of different concentrations of CS-AgNPs is presented in Fig. 7. which showed a dose-dependency. DPPH antioxidant assay is based on the ability of 1, 1 – diphenyl-2-picryh hydrazyl, a stable free radical to decolourize in the presence of CS-AgNPs. The DPPH radical contains an odd electron, responsible for the absorbance at 517 nm and also for a visible deep purple colour. The donation of electron from CS-AgNPs to the DPPH led to its decolourization, The intensity of the colour was quantitatively measured from the changes in absorbance. Variations in colour intensity of the prepared concentrations were observed in this study similar to reports for various AgNPs [44, 61]. Free radical and reactive oxygen species are the main cause of several disorders in humans, which is generated as an imbalance between formation and neutralization of prooxidants resulting in oxidative stress. Free radicals play a significant role in diverse diseases such as cardiovascular conditions, cancer, diabetes, inflammatory diseases, and early aging [62, 63]. The antioxidant compounds present in

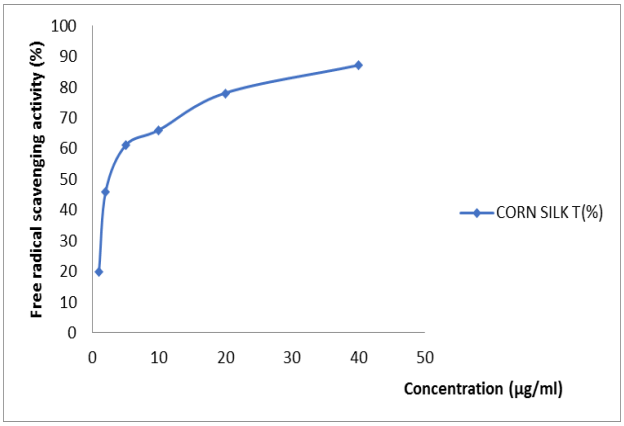

Fig. 7: Antioxidant activity of corn silk extract against DPPH.

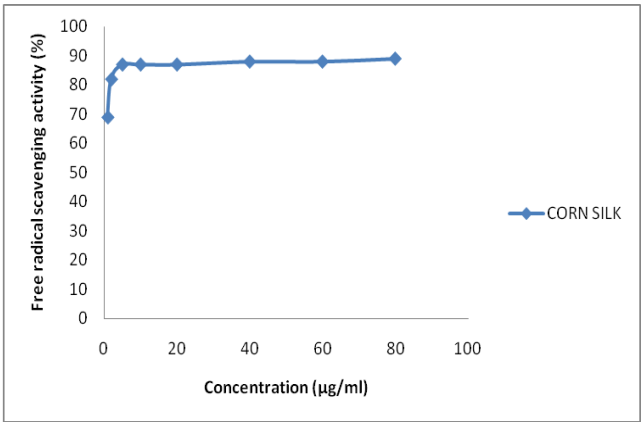

Fig. 8: Antioxidant activity of CS-AgNPs against H_2O_2

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Table 1: Cytological effects of CS-AgNPs on *Allium cepa* after 24 hours of treatment

Conc. (µg\ml)	No of dividing cells	Mitotic index	Mitotic inhibition	Prophase	Metaphase	Anaphase	Telophase	SK	MD	DA	DM	VC	FG	CM	Total No of aberrant cells	% aberrant Per dividing cells	% aberrant per cells scored
Control	485	9.70		151	115	116	103					-					
$AgNO_3$																	
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	1	-			3	0.79	0.06
1	289	5.78	40.41	84	70	72	63	1		2	1	-			4	1.38	0.08
10	254	5.08	47.62	76	63	58	57	2		1	1	-			4	1.57	0.08
100	232	4.64*	52.16	67	57	53	55	2			1	-	2		5	2.15	0.10
CS Extract																	
0.01	498	9.96	-2.68	136	126	123	113	1							1	0.20	0.02
0.1	485	9.70	0.00	133	126	120	106			1					1	0.20	0.02
1	460	9.20	5.15	131	121	113	95	2							2	0.43	0.04
10	296	5.92	38.97	82	76	71	67			1	1				2	0.67	0.04
100	169	3.38*	65.15	46	42	40	41	3							3	1.77	0.06
CS-AgNPs																	
0.01	496	9.92	-2.27	158	116	119	103			1					1	0.20	0.02
0.1	480	9.60	1.03	150	114	113	103			2				1	3	0.63	0.06
1	456	9.12	5.98	138	112	111	95	2		1		1			4	0.87	0.08
10	277	5.54	42.89	85	70	63	59		1	2				2	5	1.81	0.10
100	138	2.76*	71.55	43	36	29	30	3						2	5	3.89	0.10

SC: Sticky chromosome, DM: Disturbed metaphase; CM: C-Mitosis, VC: Vagrant chromosome. DA: Disturbed anaphase and FG: Fragmentation

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Table 2: Cytological effects of CS-AgNPs on *Allium cepa* after 48 hours of treatment

Conc. (µg\ml)	No of dividing cells	Mitotic index	Mitotic inhibition	Prophase	Metaphase	Anaphase	Telophase	SK	MD	DA	DM	FG	CM	VC	Total No of aberrant cells	% aberrant Per dividing cells	% aberrant per cells scored
Control $AgNO_3$	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	1.11	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	2	-	7	2.54	0.14
100	229	4.58*	55.01	63	54	54	58	4			3		1	-	8	3.49	0.16
Corn silk Extract																	
0.01	439	8.78	11.39	121	113	104	101	1			1				2	0.45	0.04
0.1	387	7.74	23.97	110	101	95	81	2							2	0.51	0.04
1	289	5.78	43.22	81	73	70	65	3							3	1.03	0.06
10	179	3.58*	64.83	51	44	44	40	1		3					4	2.23	0.08
100	100	2.00*	80.35	28	26	24	22	3		2					5	5.00	0.10
CS-AgNPs																	
0.01	330	6.60	35.16	95	86	73	76		1	3	3				7	3.04	0.14
0.1	318	6.36	37.52	88	83	77	70	2			1		2		5	2.29	0.10
1	295	5.90	42.0.4	81	79	75	60	5			1	1		1	8	4.10	0.16
10	144	2.88*	71.71	44	33	35	32	4		1		4			9	6.25	0.18
100	103	2.06*	79.76	27	29	23	24	5					2	1	8	7.77	0.16

Mitotic index less than half of the value of the control

SC: Sticky chromosome, DM: Disturbed metaphase; CM: C-Mitosis, VC: Vagrant chromosome. DA: Disturbed anaphase and FG: Fragmentation

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Table 3: Cytological effects of CS-AgNPs on *Allium cepa* after 72 hours of treatment

Conc. (µg\ml)	No of dividing cells	Mitotic index	Mitotic inhibition	Prophase	Metaphase	Anaphase	Telophase	SK	MD	DA	DM	FG	CM	VC	Total No of aberrant cells	% aberrant Per dividing cells	% aberrant per cells scored
Control	490	9.80	-	155	115	114	106							-			
$AgNO_3$																	
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	1	2	-	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
Corn silk Extract																	
0.01	295	5.90	39.80	83	73	72	67	4							4	1.35	0.08
0.1	242	4.84*	50.61	66	60	61	55			3	1				4	1.65	0.08
1	183	3.66*	62.65	54	47	42	40	4		1					5	2.73	0.10
10	138	2.76*	71.84	41	36	32	29	5							5	3.62	0.10
100	98	1.96*	80.00	27	25	24	22	6							6	6.12	0.12
CS-AgNPs																	
0.01	340	6.80	30.61	86	85	84	85	6		1		1			4	2.85	0.04
0.1	203	4.06*	58.57	60	50	51	42	3		3	1	2			4	3.88	0.08
1	163	3.26*	66.73	44	41	42	36	3		2		2			5	5.62	0.10
10	125	2.50*	74.49	38	32	31	24	3				2			5	7.46	0.10
100	89	1.78*	81.84	27	25	23	14	4			1	1			6	10.52	0.12

SC: Sticky chromosome, DM: Disturbed metaphase; CM: C-Mitosis, VC: Vagrant chromosome. DA: Disturbed anaphase and FG: Fragmentation

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Table 4: Comparison of Effects of $AgNO_3$, Corn Silk Extract and its Nanoparticles on *Allium cepa* Root Growth

Concentration (µg/ml)	$AgNO_3$				Corn silk Extract only				CS-AgNPs			
	Total (N)	Mean root length+SE	%Mean root length	%Root inhibition control	Total (N)	Mean root length+SE	%Mean root length	%Root inhibition control	Total (N)	Mean root length+SE	%Mean root length	%Root inhibition control
Control	255	3.58^{a}±0.92	100	-	255	3.58^{a}±0.92	100	-	255	3.58^{a}±0.92	100	-
0.01	239	2.67^{b}±0.76	74.58	25.42	150	2.65^{b}±0.79	74.02	25.98	197	3.32^{a}±0.07	92.74	7.26
0.1	242	2.43^{c}±0.61	67.87	32.12	149	2.22^{c}±0.81	62.01	37.99	180	2.80^{b}±0.06	78.21	21.79
1.0	169	2.04^{d}±0.63	56.98	43.02	128	1.94^{d}±0.75	54.19	45.81	192	2.18^{c}±0.04	60.89	39.11
10.0	213	1.39^{e}±0.43	38.83	61.17	116	1.03^{e}±0.51	28.77	71.23	147	0.66^{d}±0.07	18.44	81.56
100	124	$0.35^{f}_{\pm}$0.02	9.78	90.22	119	$0.63^{f}_{\pm}$0.33	17.60	82.40	112	$0.22^{e}_{\pm}$0.01	6.15	93.85

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

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the CS-AgNPs convert DPPH radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom. The colour change from the purple of DPPH radical to the pale yellow of the reduced form of DPPH allows the spectrophotometric determination of the antioxidant activity. Therefore, CS-AgNPs revealed the presence of antioxidant potential.

3.5.2 Hydrogen peroxide (H_2O_2) scavenging activity

The CS-AgNPs scavenged hydrogen peroxide (Fig. 8) in a dose-dependent manner. This effect may be due to exchange interactions between the unpaired electrons of the free radicals and conduction band electrons of the metal nanoparticles. Such effect has been reported for gold nanoparticles [64]. A dose-dependent activities of 69—89% was obtained for the corn silk AgNPs at concentrations of 1-80 µg/ml in this study similar to those previously reported [61, 65, 66]. Accumulation of H_2O_2 often leads to the development of oxygen free radicals which causes a great damage to the cell membrane. The presence of hydrogen peroxide inside a cell at a low concentration stimulates the dissolution of AgNPs and produces more oxidative stress [67]. Therefore, CS-AgNPs play an important role in absorbing and neutralizing free radicals.

3.6 Cytogenotoxic effects of CS-AgNPs on *Allium cepa*

The effects of the $AgNO_3$, CS-extract, and CS-AgNPs at 24, 48, and 72 h are shown in Tables 1, 2 and 3, respectively. The number of dividing cells obtained at 24 h were lower at all concentrations of $AgNO_3$ compared to corresponding concentrations of CS-extract and CS-AgNPs, except at 100 µg/ml (Table 1). The values obtained for CS-AgNPs were in turn lower compared with CS -extract at all concentrations. However, at 48 h (Table 2) and 72 h (Table 3), the number of dividing cells was higher in $AgNO_3$ compared to CS-extract and CS-AgNPs, while that of CS-extract was also higher than those of CS-AgNPs. The Mitotic index (MI) obtained for the control at each period of evaluation was higher than those of different concentrations in each of the three treatments. The MI value obtained at 100 µg/ml was lower than half the value of the control at 24 h of $AgNO_3$, CS- extract and CS-AgNPs (Table 1), while at 48 h only the value obtained at 100 µg/ml of $AgNO_3$, 10 and 100 µg/ml of Cs-extract and CS-AgNPs were less than half of the control (Table 2). All concentrations at 72 h had the MI values lower than half of the values obtained for the control, except 0.01 and 0.1 of $AgNO_3$ as well as 0.01µg/ml of CS-extract and CS-AgNPs (Table 3),. This shows that the toxicity of the treatments increased as the period of exposure increases. The toxicity observed was also dose-dependent within each of the treatments.

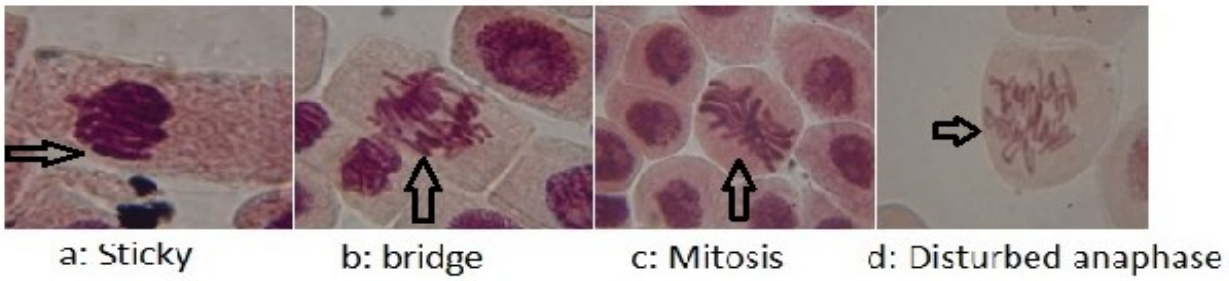

Fig. 9: Chromosomal aberrations observed in *A. cepa* cells exposed to corn silk mediate silver nanoparticles.

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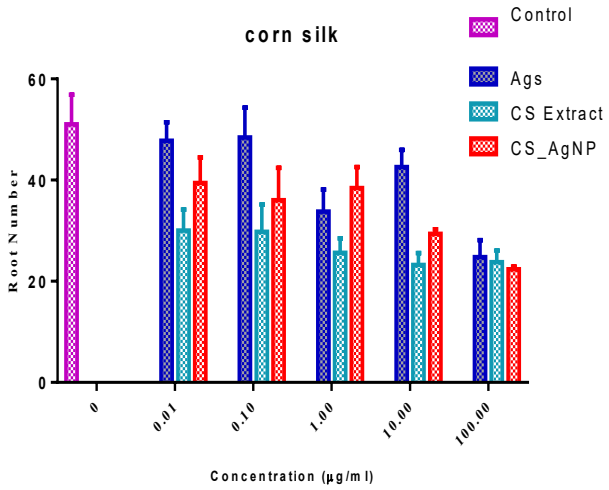

Fig. 10: Root numbers of *Allium cepa* exposed to $AgNO_3$, corn silk extract and CS-AgNPs

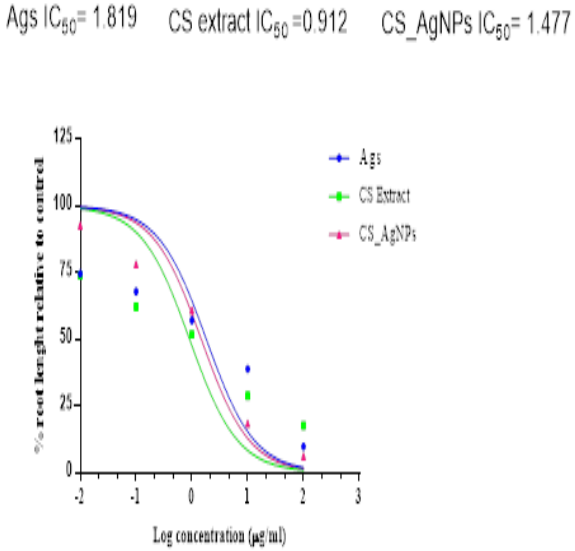

Fig. 11: Effective concentration of $AgNO_3$, CS-extract and CS-AgNPs on *Allium cepa* root length

Toxicity is ascertained whenever the value of MI in at least two consecutive concentrations is less than half of the value of the control as stated in the previous reports where *Allium cepa* assay was employed [30, 31, 32, 68, 69].

The mitotic inhibition observed with the various concentrations of the treatments throughout the exposure periods showed a dose-dependent increase in toxicity, with the highest toxicity observed at 100 µg/ml. However, at 0.01 of CS-extract and CS-AgNPs there was more cell proliferation than the control.

The trend as observed for various stages of division in the control and most higher concentrations of all treatments revealed the highest number of prophase which decreased through to the telophase. A dose-dependent decrease in the number of mitotic stages was observed in all treatments throughout the periods of exposure (Tables 1, 2, and 3). Except in the control, various concentrations of each treatment induced different proportions of chromosomal aberrations. In most cases, the highest percentage of aberrant cells were obtained at the highest concentration of the treatments (Table 1, 2, 3). Aberrations observed were sticky chromosome, disturbed metaphase, c-mitosis, vagrant chromosome, disturbed anaphase, and fragmentation with none been dominant (Figure 9 and Tables 1,2,3).

The mean root number obtained per concentration in each treatment at 72 h exposure period is shown in Table 4. The highest root number was observed in the control. A dose-dependent reduction of root number was observed in each of the $AgNO_3$, CS-extract, and CS-AgNPs. It was however observed that the number of the roots in Cs-$AgNO_3$ though lower than that of control, was higher than those of CS-extract at their corresponding concentrations, except 100 µg/ml and found lower than those of $AgNO_3$, except at 1.0 µg/ml. Variation in root numbers within and between treatments is as shown in figure 10. The percentage root growth of the treated onions relative to the control revealed a dose-dependent significant decrease in each of the treatments. The values obtained at all concentrations in each case were significantly different from that of the control ($P \leq 0.05$). The root growth inhibitory activity of the three treatments which serve as indicator of their toxicity was in the order of $AgNO_3$< CS

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-AgNPs< CS- extract (Figure 11). Inhibitory effects on root growth had been reported as an efficient means of ranking toxicity, and efficiency of the *Allium cepa* assay in evaluating the toxicity of various substances had been reported for chemicals [70, 71], pesticide [32, 68, 72], wastewater [33, 73, 74] and aqueous extracts of vegetables [75, 76].

5. Conclusion

This study revealed that corn silk extract mediated the synthesis of silver nanoparticles observed via changes in colour with absorbance peak at 436 nm. The CS-AgNPs were polydispersed and spherical shaped with the size range of 8.49–45.78 nm. Furthermore, the particles were crystalline, showing the properties of a face-centered cubic structure. The biosynthesized AgNPs displayed good larvicidal activity on Anopheles mosquito larvae, where 100% mortality was achieved within 12 h of exposure. The CS-AgNPs displayed anticoagulant and thrombolytic activities which might be useful for application in nanomedicine for prevention of blood coagulation. In addition, DPPH and Hydrogen peroxide free radical scavenging ability of the CS-AgNPs could also be of benefit. Although CS-AgNPs showed cytotoxicity potential and also induced chromosomal aberrations, its cell division inhibitory potential can be explored as an anti -cancer agent. Caution is however required in its applications and proper guidance be sought before being used in the environment.

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