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Biomedical Applications of *Chasmanthera dependens* stem extract mediated silver nanoparticles as Antimicrobial, Antioxidant, Anticoagulant, thrombolytic, and Larvicidal agents

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Biomedical Applications of *Chasmanthera dependens* stem extract mediated silver nanoparticles as Antimicrobial, Antioxidant, Anticoagulant, thrombolytic, and Larvicidal agents

Abstract

The stem extract of *Chasmanthera dependens* was used in the biofabrication of silver nanoparticles (AgNPs) in this study. The AgNPs was characterised using UV-visible spectroscopy, Field Emission Scanning Electron Microscope (FESEM), EDX, and the Fourier Transform Infrared Spectroscopy (FTIR). Antibacterial, antioxidant, anticoagulant, thrombolytic and larvicidal activities of the biosynthesised nanoparticles were carried out. There was a peak at 418 nm with a strong silver peak observed around 2.7KeV when the EDX analysis was carried out. The FESEM showed a large number of cubically shaped nanoparticles with sizes ranging from 24.53 to 92.38 nm. CDE-AgNPs was effective against *Klebsiella pneumoniae* at 80 µg/ml and 100 µg/ml concentrations while little or no inhibition observed with other bacterial isolates used. CDE-AgNPs displayed potent activity with LC₅₀ and LC₉₀ of 7.15 µg/ml and 20.86 µg/ml respectively against the *Aedes aegypti* mosquito larva after 1hr of exposure. The synthesised nanoparticles displayed scavenging activity of DPPH at all concentrations. High anticoagulant efficacy was displayed, while 45.61% clot lysis was observed. This study therefore demonstrated the effectiveness of biosynthesised *Chasmanthera dependens* stem extract silver nanoparticles as antimicrobial, antioxidant, anticoagulant, thrombolytic and larvicidal agents.

Keywords

Chasmanthera dependens, silver nanoparticles, biosynthesis, biomedical applications

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1. Introduction

Nanotechnology is a wide subject area dealing with various disciplines including chemistry, biology, engineering and physics. *Nano* is a Greek word meaning ‘dwarf’ with particle size ranging from one to one hundred nanometres [1]. The word *nano* connotes ‘one billionth of a meter’ [2,3]. Nanotechnology entails synthesising particles which differ in shape, size and morphology [1]. These particles, being little in size, possess a large surface area to volume ratio and hence display unique optical, magnetic and electrical properties relative to their bulk material [4]. This area possess the potential to transform several segments and is being rapidly developed to allow inter-diversity between engineering, materials and life sciences [5]. In nanotechnology, the biological fabrication of nanoparticles is one of the most thriving areas of interest [6].

Recently, the uses of plants in synthesising nanomaterials have been frequently reported due to their numerous usefulness in various fields and their physico-chemical properties. Silver nanoparticles most especially have been fabricated from natural sources and studied extensively [7–12]. Silver has long been used to prevent and kill chronic wound infection due to their anti-inflammatory and antimicrobial abilities [13].

Some plant parts including the fruit, root, peel, seed, flowers or leaves have been reportedly utilized in synthesising silver nanoparticles of various shapes. Stem as a source for nanoparticles synthesis is gaining grounds recently. Shameli et al. reported synthesising nanoparticles from the stem methanolic extract of *Callicarpa maingayi* [14]. Also, *Cissus quadrangularis* stem extract has been reportedly used in synthesising nanoparticles [15]. Functional groups like the amine, carboxyl and phenolic compounds which are present in the stem extracts are responsible for reducing silver ions. This study is, therefore, directed at utilizing *Chasmanthera dependens* stem extracts for the synthesis of silver nanoparticles.

C. dependens (Hoschst), mostly alluded to as *Chasmanthera*, are of the family Manispermaceae. *C. dependens* has been studied to have a lot of medicinal uses. Okiei et al. [16] evaluated the antimicrobial bioactivity of its stem extract and it was found active against a host of pathogenic organisms. The analgesic and anti-inflammatory activity of the dried leaves'

methanolic extract on laboratory animals have also been reported [17].

Since nanotechnology entails fabricating, manipulating and characterizing particles less than 100 nm and these particles reportedly possess novel and unique characteristics [18], the stem extract of *C. dependens* was used in synthesising nanoparticles. Several biomolecules such as alkaloids, tannins, phenolics, saponins and vitamins which have been reportedly present in the stems of *C. dependens* [16], could be involved in the reduction and stabilization of silver ions. This has motivated the use of *C. dependens* stem extract for the biosynthesis. In this study, the antimicrobial, antioxidant, anticoagulant, thrombolytic and larvicidal properties of the biosynthesized silver nanoparticles were evaluated.

2. Materials and methods

2.1. Sample collection

The stems of *C. dependens* were purchased from Bere Market, Ibadan, Nigeria. It was brought down to the Microbiology Laboratory, Babcock University for further processing. The stem was chopped into smaller pieces and then blended into powdery form before storage at ambient temperature in air-tight container.

2.2. Collection of clinical isolates

For the antimicrobial assay of the synthesized AgNPs, four clinical bacterial isolates were collected from LAUTECH Teaching Hospital, Ogbomoso, Nigeria. They include 3 Gram negative organisms (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*) and 1 Gram positive organism (*Staphylococcus aureus*).

2.3. Collection of larva for the in vitro larvicidal study

The *Aedes aegypti* larvae that were used for this study were gotten from a ditch located on the university campus, Babcock University, Ilishan-Remo. A large covered container was used to collect the water sample containing the larvae. The target mosquito larva used for this study was identified by a medical entomologist. It was the 4th instar larva of the *A. aegypti* mosquito which was separated into distinct compartment, while the rest were discarded.

2.4. Collection of blood for the anticoagulant and thrombolytic study

Approximately 6 ml of veinous blood used for both anticoagulant and thrombolytic *in vitro* study were drawn from a healthy volunteer who had not been on any anticoagulant therapy before.

2.5. Preparation of extract

The aqueous extract of *C. dependens* stem used for this study was prepared according to the methods of Lateef et al. [19]. Hence, the extract was deepened in a 100 ml of distilled water, while 1 g of the extract was suspended. Subsequently, this was followed by heating for 1 h at 60 °C in the water bath. The extract was further filtered and the resulting solution was subjected to centrifugation for 20 min at 4000 rpm and the supernatant that resulted was used for further experiment.

2.6. Biogenic synthesis

The biogenic synthesis of the AgNPs was carried out by introducing 1 ml from the prepared extract into 40 ml of 1 mM AgNPs and the observation for colour change followed suit. The whole synthesis which took approximately 2 h was carried out at room temperature [19].

2.7. Characterisation

The AgNPs formation was first established by characterisation using the UV–visible spectrophotometer (Cecil, USA). The absorbance spectrum was measured at 190–900 nm. Surface structure of the AgNPs was characterised using Shimadzu FTIR spectrometer, model 8400S (Shimadzu, Japan) and KBr as diluent pressed pellets was used for the measurement. FTIR spectrum was measured between 4000 and 400 cm^{-1} wavelength. In order to estimate the presence and ratio of the elements in the particles, the EDX of the nanoparticles was carried out with the EDX Silicon-drift detector (X-MaxN, Oxford Instruments, UK). The presence, size alongside the structural morphological characteristics of the synthesized AgNPs was analysed with the FESEM. Powder samples were sonicated in ultrasonic probe sonicator in ethanol medium to have maximum deagglomeration of the particles before imaging. Surface morphologies of the synthesised particle samples were observed with a FESEM (Lyra 3, Tescan, Czech Republic) at an accelerating voltage of 20 up to 30 kV. Open-source

software, Image J, was used to independently analyse image attributes and determine nanoparticles size [20].

2.8. Antimicrobial activity

To assess the efficacy of the biosynthesized CDE-AgNPs against some selected isolates of clinical importance, the agar well diffusion method was employed. An 18 h-old culture of each of the organisms prepared overnight in peptone broth was used to inoculate an already prepared plate of Mueller Hinton agar. Holes were bored on the inoculated plate using a sterile 6 mm cork borer and the holes were labelled 10, 20, 40, 60, 80 and 100 $\mu\text{g/ml}$ respectively. Varying concentrations of 100 μl of the synthesized AgNPs was then introduced into the corresponding holes. Control experiments were set up with CDE, silver nitrate and distilled water. The plates were incubated for 18 h at 37 °C after which the zone of inhibition was read [21]. The agar well diffusion test was performed in triplicate.

2.9. Larvicidal efficacy of the nanoparticles

To assay the efficacy of the AgNPs against the 4th instar larva *A. aegypti*, the WHO recommended guideline was used [22]. A 96-hole microtitre plate was used. Five larvae were introduced into each of the 5 holes labelled 20, 40, 60, 80 and 100 $\mu\text{g/ml}$ respectively. Then, 300 μl of each of the concentration of AgNPs was dispensed into each of the corresponding holes as labelled. A control experiment was set up in which sterile distilled water was introduced to the larvae. The test was carried out in triplicate and readings taken after 12 h of treatment. This was subsequently followed by statistical evaluation of the percentage mortality and Probit analysis for the computation of LC_{50} and LC_{90} .

2.10. Antioxidant activity

To evaluate the efficacy of the AgNPs to mop up free radicals, 4 ml methanolic solution of 0.1 mM DPPH (Sigma Aldrich) was introduced into each of 4 bijou bottles labelled 10, 20, 40, and 60 $\mu\text{g/ml}$ respectively. Thereafter, 1 ml of each graded concentration was introduced into the corresponding bottles as fast as possible, shaken and kept in a dark box at room temperature for 30 min. After 30 min of incubation in the dark, the absorbance was read at 517 nm against absolute methanol (blank) [23]. The antioxidant activity of the biosynthesized AgNPs was calculated as follows:

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

2.11. Anticoagulant activity

The efficacy of the biosynthesized AgNPs as anti-coagulants was estimated as described by Lateef et al. [7]. Exactly 0.5 ml of the collected fresh healthy blood was dispensed into each of two Eppendorf tubes. One of the Eppendorf tubes was introduced with 0.1 ml of the synthesized AgNPs. The other Eppendorf tube served as negative control as nothing was introduced; however, a positive control in which 0.5 ml of blood was introduced into EDTA bottle was also set up. The reaction mixtures were held for 1 h at room temperature after which it was observed microscopically under the photomicrograph.

2.12. Thrombolytic activity

To determine the thrombolytic activity of the synthesised AgNPs, 3 pre-weighed Eppendorf tubes were dispensed with 0.5 ml of blood and kept in the incubator at 37 °C for 30 min. After the formation of clot, the serum was completely removed without disturbing the test. The tubes were then reweighed to estimate the weight of the clotted blood. One of the tubes was treated with 0.1 ml AgNPs to serve as the experimental set up, while the control experiment was treated with 0.1 ml AgNO₃ and 0.1 ml of the plant extract. The reaction mixtures were further incubated at 37 °C for another 1 h. The tubes were brought out after this period and inverted to remove the lysed clot via decantation. Finally, the tube was reweighed to estimate the weight of blood lysed and calculate thrombolytic activity. This method was as earlier described [7].

3. Results and discussion

3.1. Synthesis and characterization

An observation of the colour change in the reacting solution revealed the reduction of the silver nitrate into AgNPs. The solution, initially colourless, within 10 min, formed a stable dark brown colour (Fig. 1). This colour change has been recorded to be as a result of the excitation of surface plasmonic vibrations of silver nanoparticles [24–26].

The UV–vis spectrum of the synthesized AgNPs peaked at 418 nm as shown in Fig. 2. This greatly

correlates with previous findings from various researchers. Kota et al. [27] in their synthesis of AgNPs with the leaf extract of *Rumex acetosa* reported that the UV–vis spectrum peaked at approximately 448 nm. Kannan et al. [28] also had similar observation when *Ganoderma lucidum* extract was used in the synthesis of silver nanoparticles.

During nanoparticles synthesis, the use of plants extracts introduces organic functional groups such as carbonyls, amide and hydroxyls on the surface of the NPs. These functional groups are often characterized using FTIR spectroscopy (Fig. 3). The NPs presents peaks at 3454 cm⁻¹ assigned to stretching vibration of –NH₂ (amide I) and/or –OH of phenolic compounds [29]. The presence of infrared bands at 2426 cm⁻¹, and 2360 cm⁻¹ are due to the atmospheric CO₂ absorption [30,31]. This adsorption of CO₂ suggested that the nanoparticles may be useful in atmospheric carbon dioxide removal; this however needs to be verified. The 1384 cm⁻¹ peak has also been linked to in plane bending of alkenes and aromatics [32]. The 2019 cm⁻¹ peak is that of C≡C stretching, while the 1637 cm⁻¹ peak is that of –N–H bend [33,34]. The NPs surface carbonate vibrational mode is at 873 cm⁻¹ and Ag–O stretching modes are observed at 669 cm⁻¹ and 420 cm⁻¹ [35]. The 617 cm⁻¹ peak is probably that of sulfonate esters.

EDX analysis (Fig. 4) was used to verify the presence of elemental silver in the synthesised AgNPs. A strong silver signal peak was observed around the 2.7 keV, while weak silver signal peaks were noted at the 2.9 keV, 3.0 keV and 3.2 keV. Rao and Tang [25] in their synthesis of AgNPs with leaf extract of *Eriobotrya japonica* reported signal peak of silver at 3.0 keV. The optical absorption peak approximately at 2.983 keV was equally earlier reported by Jagtap and Bapat [36]. Other elemental peaks, including Na, Si, Mg, K, O, C and Cl were likewise spotted. The O and C are probably from the extract, while others are impurities.

FESEM which revealed the morphology and size of the synthesised silver nanoparticles (Fig. 5) showed a large number of cubically shaped nanoparticles with sizes ranging from 24.53 to 92.38 nm. The interactions of nanoparticles with stabilisers and inductors around them have been known to influence their shape [37], which in turn influenced their reaction rate [38]. In a previous paper by Poinern et al., the presence of Ag nanocubes 90 min after the initial reduction process, with sizes of the cubes ranging from 50 to 200 nm was documented [39].

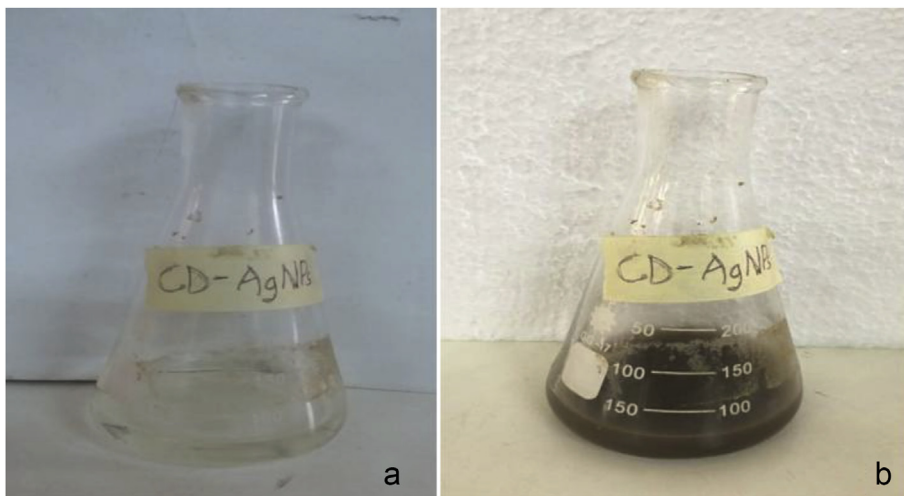


Fig. 1. Synthesis of the *Chasmanthera dependens* extract mediated silver nanoparticles (a) immediately after adding *Chasmanthera dependens* extract to the silver nitrate; (b) Formation of dark brown colouration after 30 min.

3.2. Antimicrobial activity

CDE-AgNPs displayed a minimal inhibitory activity against *S. aureus* and *E. coli* as it was only efficient at 100 $\mu\text{g/ml}$ concentration. CDE-AgNPs at both 80 and 100 $\mu\text{g/ml}$ concentrations were, however, effective against *K. pneumoniae* and *P. aeruginosa*. CDE-AgNPs gradient concentrations from 10 to 70 $\mu\text{g/ml}$ were totally ineffective against the bacterial

isolates. The antibacterial activity could be because of the limit of the CDE-AgNPs to hold fast to the bacterial cell layer at high fixations and enter quick into the cytoplasm which create basic changes in the cell wall, and subsequently the inhibition of the organism [8]. Different researchers have reported the various antibacterial efficacy of biosynthesized AgNPs against some clinical isolates [18,23,28,40] (see Table 1) (see Fig. 6).

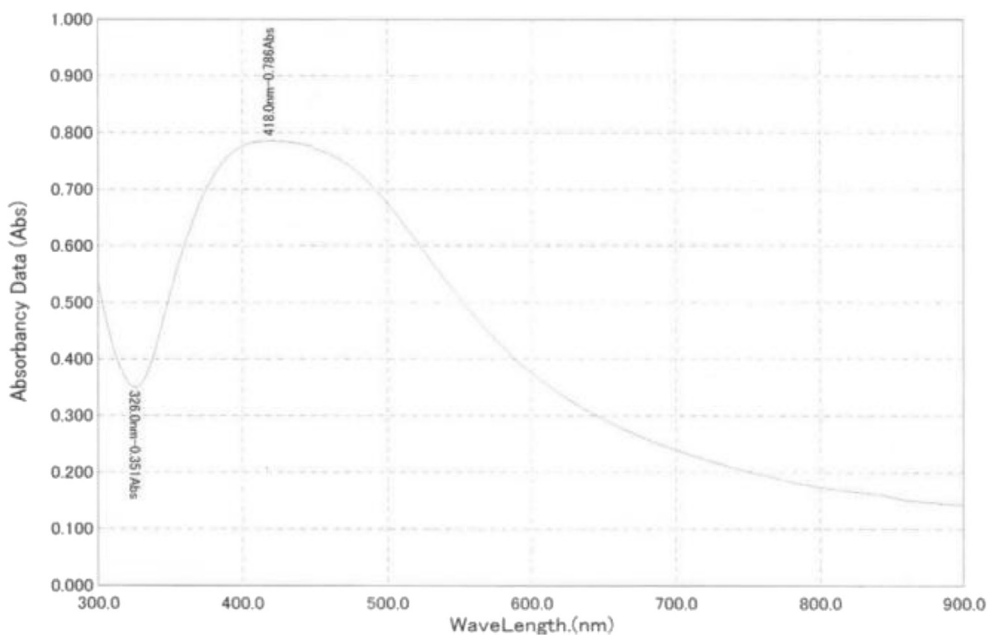


Fig. 2. The UV–Visible spectrum of the biosynthesized CDE-AgNPs.

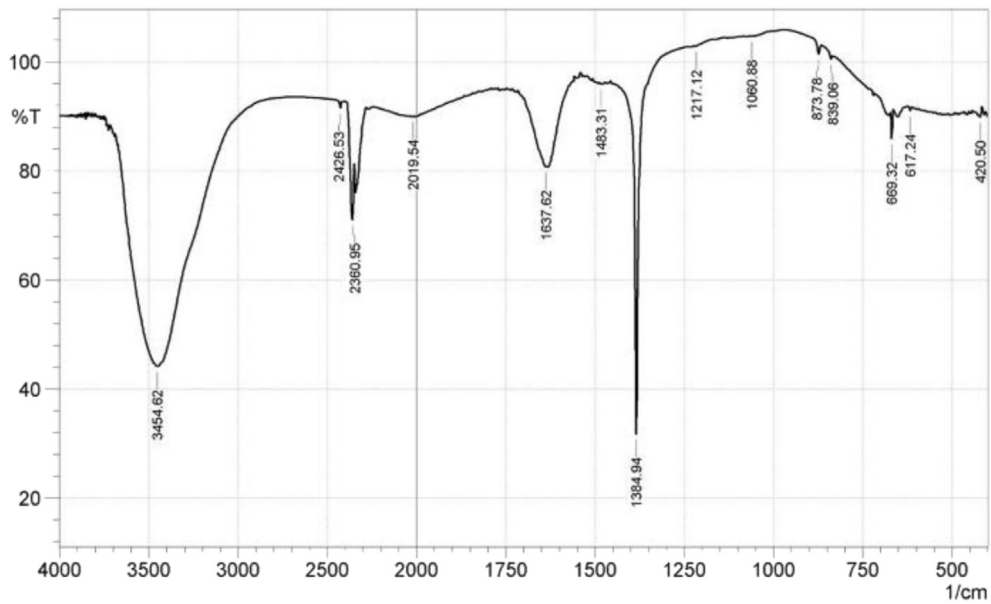


Fig. 3. The FTIR spectrum of the biosynthesized CDE-AgNPs.

However, the resistance of certain organisms to biosynthesised AgNPs has also been recently reported. Another report of the evaluation of the antimicrobial activity of AgNPs synthesised from *Artocarpus heterophyllus* Lam. seed extract against some bacterial isolates recorded no zone of inhibition against some Gram negative and Gram positive bacteria [36]. This resistance could be caused by Flagellin, a component

of the bacterial flagellum, which makes the silver nanoparticles clump together and hence make them lose their efficacy against the bacteria [41].

3.3. Larvicidal activity

CDE-AgNPs displayed potent activity against *A. aegypti* mosquito larva at concentrations of

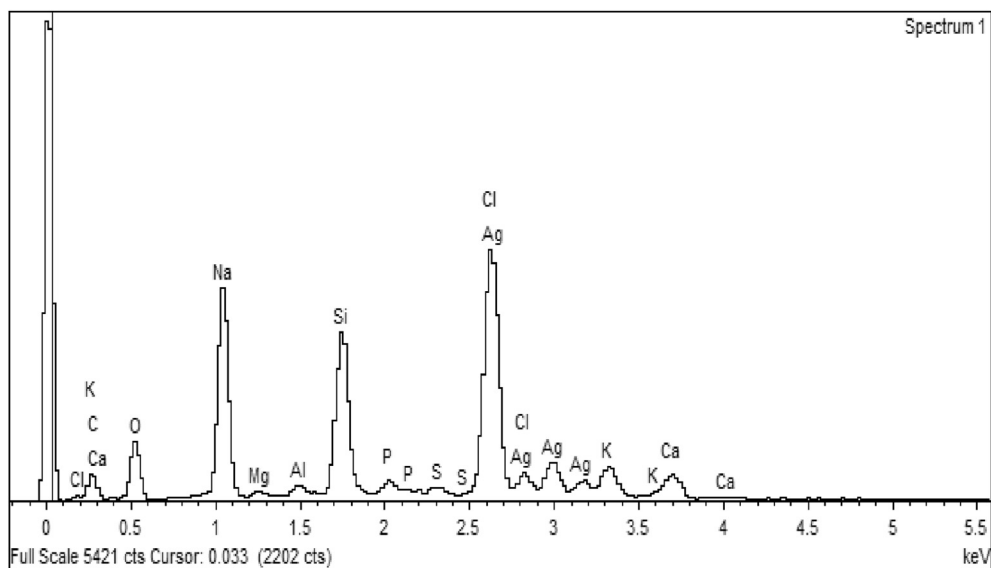


Fig. 4. Energy dispersive spectrum of the biosynthesized CDE-AgNPs.

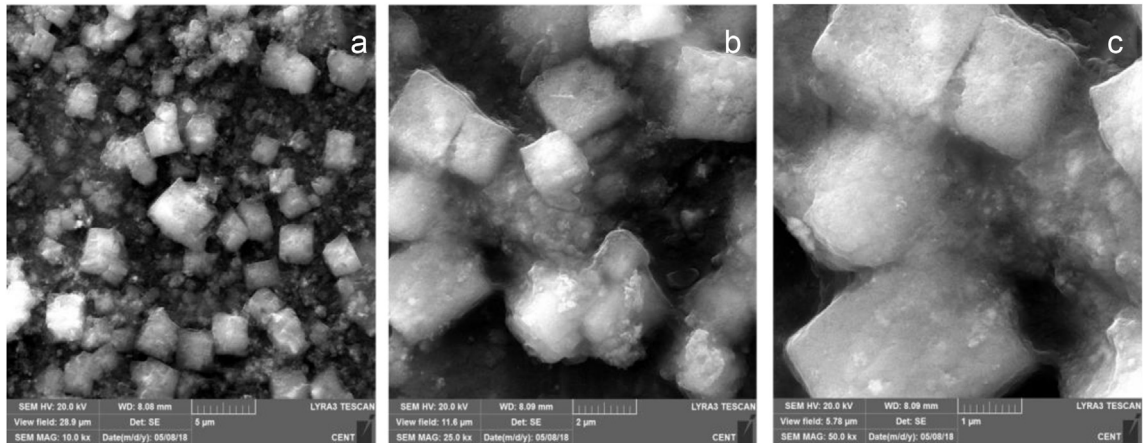


Fig. 5. Field Emission Scanning Electron Micrograph of the biosynthesized CDE-AgNPs at (a) 10kx magnification (b) 25kx magnification (c) 50kx magnification.

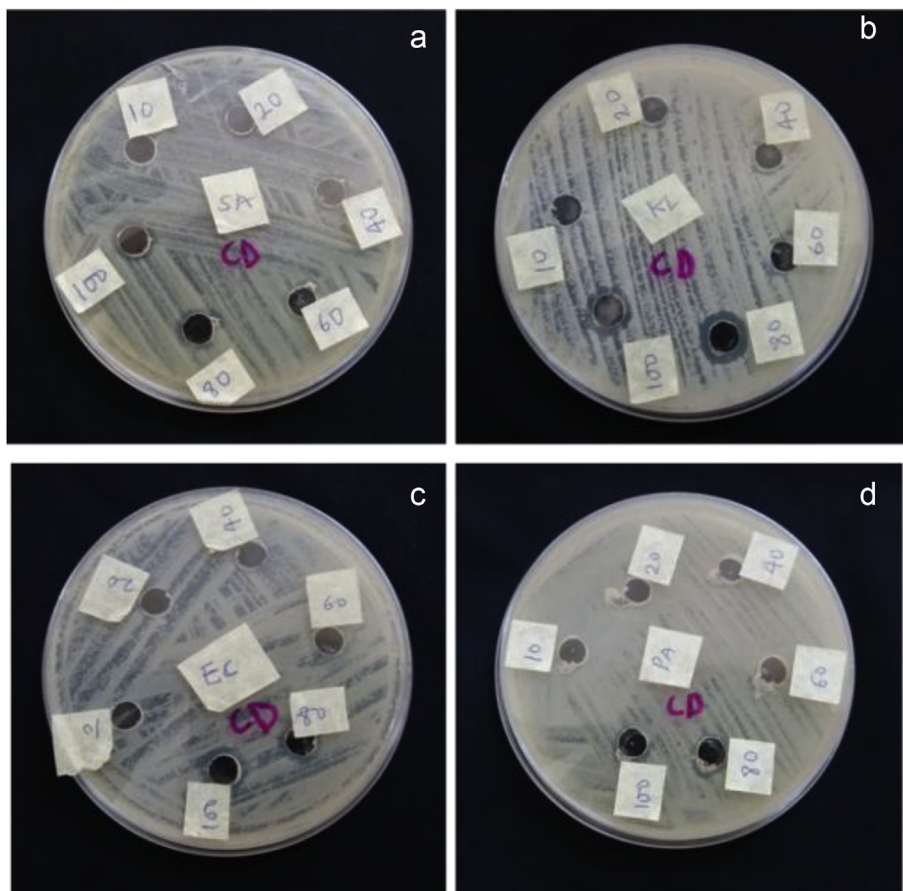


Fig. 6. The antibacterial activities of the biosynthesized CDE-AgNPs (a) against *Staphylococcus aureus* (b) against *Klebsiella pneumoniae* (c) against *Escherichia coli* (d) against *Pseudomonas aeruginosa*.

Table 1
Zone of inhibition of the biosynthesized CDE-AgNPs against some selected pathogens.

Isolate	Mean Zone of Inhibition (mm) \pm standard deviation (SD)						AgNO ₃	CDE	Distilled H ₂ O
	AgNPs								
	10 μ g/ml	20 μ g/ml	40 μ g/ml	60 μ g/ml	80 μ g/ml	100 μ g/ml			
<i>Staphylococcus aureus</i>	NZ	NZ	NZ	NZ	NZ	8.0 \pm 0.1	6.2 \pm 0.2	NZ	NZ
<i>Klebsiella pneumoniae</i>	NZ	NZ	NZ	NZ	14 \pm 0.2	12 \pm 0.1	6.4 \pm 0.1	NZ	NZ
<i>Pseudomonas aeruginosa</i>	NZ	NZ	NZ	NZ	9.0 \pm 0.1	8.0 \pm 0.2	6.1 \pm 0.1	NZ	NZ
<i>Escherichia coli</i>	NZ	NZ	NZ	NZ	8.4 \pm 0.2	10 \pm 0.1	6.3 \pm 0.1	NZ	NZ

20–100 μ g/ml within 2 h as indicated in Fig. 7. At 80 and 100 μ g/ml concentrations, CDE-AgNPs effect was high, totally killing the mosquito larva. After 1 h of exposure to the 50 and 90% lethal concentrations (LC₅₀ and LC₉₀) of 7.15 μ g/ml and 20.86 μ g/ml respectively which were gotten through probit analysis. The larvicidal activity of the AgNPs could be because of the penetration of the particles to debilitate cell metabolism because of their attachment to enzymes and DNA. CDE-AgNPs larvicidal activities were similar to reported larvicidal activity of some AgNPs of bacterial extract origin and plants against *A. aegypti* larvae [42,43].

3.4. Antioxidant activity

The synthesized silver nanoparticles displayed a fairly good scavenging activity of DPPH at all concentrations, with activities ranging from 3.6 to 29.7% at a working concentration of 10–60 μ g/ml. The scavenging

activities of the AgNPs used in this study are comparable although less efficacious compared to those that were previously reported [23,44–46]. The free radical scavenging activity of the nanoparticle has been attributed to the functional groups of the bioreductant molecules clung to the surface of the particles.

3.5. Anticoagulant activity

A paper report in 2009 indicated the possibility of utilising AgNPs as antiplatelet and antithrombotic agents as they have innate antiplatelet properties hence preventing integrin-mediated platelet responses [47]. In this study, the anticoagulant property of CDE mediated AgNPs was evaluated. The nanoparticles displayed wonderful anticoagulant efficacy (Fig. 8) with the nanoparticles producing similar result to those produced in the presence of EDTA. Upon viewing the AgNPs-treated blood sample under the microscope, it was observed that the biconcave disc of the red blood

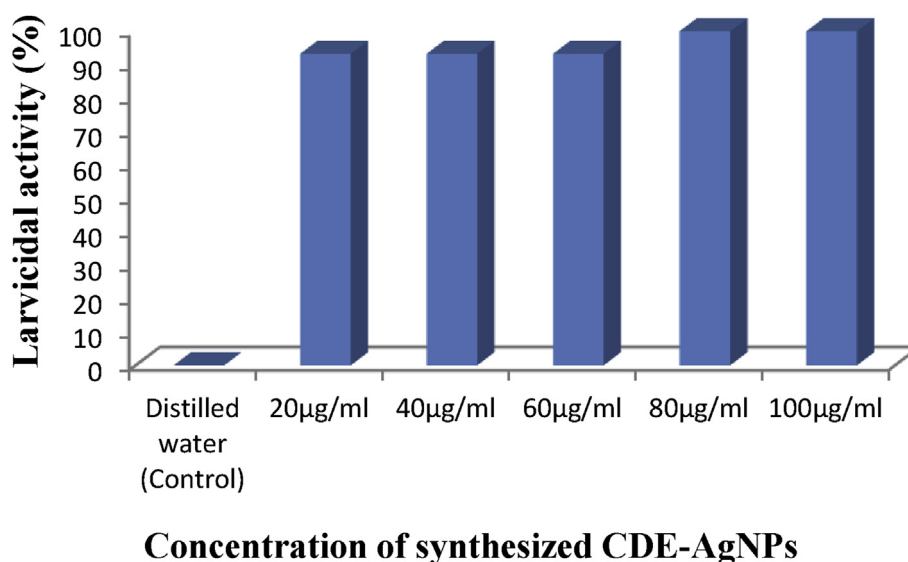


Fig. 7. Larvicidal activity of the biosynthesized CDE silver nanoparticle on the Larvae of *Aedes aegypti* Mosquito.

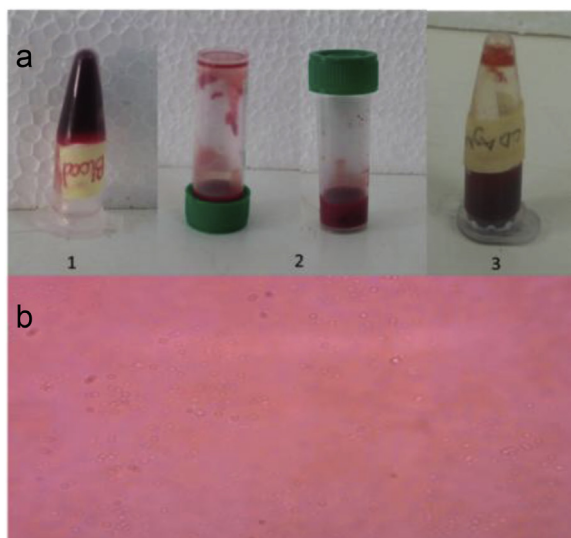


Fig. 8. Anticoagulant property of the biosynthesized CDE-AgNPs. (A) Macroscopic examination (1. clotted blood, serving as negative control; 2. blood in EDTA-no clot, serving as positive control; 3. blood + AgNPs-no clot) B. Microscopic view of blood treated with the synthesized AgNPs.

cells was not tampered with. This is similar to the results gotten when *Peltophorum pterocarpum* mediated AgNPs was evaluated for its anticoagulant property [48]. In 2016, a report on the anticoagulant activities of 3 biosynthesized silver nanoparticles (cobweb, *Cola nitida* seed shell and *Cola nitida* pod) with similar results was documented [7].

3.6. Thrombolytic activity

For the *in vitro* thrombolytic activity of the synthesized nanoparticles, the study revealed that the synthesized AgNPs showed 45.61% clot lysis as opposed to the 1.89% shown by the plant extract on the same blood sample. As earlier reported by several other researchers [16,49], the use of silver nanoparticles in the development of novel thrombolytic compounds needed to be looked into. Despite the fact that there are a few thrombolytic drugs with those acquired by recombinant DNA innovation, side effects identified with a portion of these medications that prompt further troubles have been reported [50–52].

4. Conclusion

This work has clearly revealed the usefulness of *C. dependens* stem extract as an eco-friendly and cost effective biological resources in the green synthesis of

nanoparticles. The biosynthesized nanoparticles exhibited a remarkable antibacterial, antioxidant, anticoagulant, thrombolytic and larvicidal activities. Therefore, this plant could be explored by biomedical industries for various bio resources of importance. This is the first report to the best of our knowledge on this discovery.

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