Research Article

Investigation of the Larvicidal Potential of Silver Nanoparticles against *Culex quinquefasciatus*: A Case of a Ubiquitous Weed as a Useful Bioresource

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Received 24 November 2015; Revised 26 February 2016; Accepted 1 March 2016

Academic Editor: Yogendra Mishra

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Biosynthesized silver nanoparticles (AgNPs) using *Cassia hirsuta* aqueous leaf extract were reported in this study. The synthesis was optimized by measuring various parameters such as temperature, time, volume ratio, and concentration. The surface plasmon resonance at 440 nm for 30°C and 420 nm for both 50°C and 70°C measured using the UV-Vis spectrophotometer confirmed the formation of AgNPs synthesized using *C. hirsuta* (CAgNPs). The functional groups responsible for the reduction and stabilization of the NPs were identified using Fourier Transform Infrared (FTIR). The morphology, size, and elemental composition of the NPs were obtained using scanning electron microscope (SEM), transmission electron microscope (TEM), and energy dispersive X-ray spectroscopy (EDX). X-ray diffractometer was used to identify the phases and crystallinity of CAgNPs. Crystalline spherical NPs with average diameter of 6.9 ± 0.1 nm were successfully synthesized. The thermal analysis of CAgNPs was observed from DSC-TGA. The larvicidal results against the different larva instar stage of *Culex quinquefasciatus* gave $LC_{50} = 4.43$ ppm and $LC_{90} = 8.37$ ppm. This is the first study on the synthesis of AgNPs using *C. hirsuta* and its application against lymphatic filariasis vector. Hence, it is suggested that the *C. hirsuta* synthesized AgNPs would be environmentally benign in biological control of mosquito.

1. Introduction

Mosquitoes are a well-known group of insects widely distributed worldwide. They are important vectors which transmit many dreadful diseases such as malaria, yellow fever, filariasis, dengue, and haemorrhagic fever. More than 500 million people are infected annually. In Nigeria, mosquito-borne diseases constitute a major health problem. It is statistically shown that malaria alone accounts for about 300,000 deaths from over 20 million clinical cases annually, while other mosquito-borne diseases have accounted for a lot of economic loss, social disgrace, low productivity, absenteeism, and sleeplessness [1]. *Culex* species usually breed profusely in polluted gutters, blocked drains, and other water

retention habitats containing organic matter unlike *Aedes* and *Anopheles* mosquitoes which prefer clean ground pools and man-made containers, respectively [2]. *Culex quinquefasciatus* is a vector of lymphatic filariasis, which affects 120 million people worldwide, and approximately 400 million people are at risk of contracting filariasis, resulting in an annual economic loss of US\$1.5 billion [3]. *Culex quinquefasciatus* accounts for over 70% of the mosquitoes in Nigeria. Filariasis has been shown to be a public health problem in Africa, particularly in the northern savannah and in the south-western coastal parts of Africa [4]. Infection with lymphatic filariasis, commonly known as elephantiasis, occurs when thread-like, filarial parasites are transmitted to humans through mosquitoes [5]. Larviciding is the application of chemicals

to kill mosquito larvae or pupae in the water. It is generally more effective and target-specific than applying chemicals to kill adult mosquitoes (adulticiding). In controlling mosquitoes' larvae, dichlorodiphenyltrichloroethane (DDT), organophosphate temephos, methoprene, pyrethroids, phytochemicals, and soil bacterium (*Bacillus thuringiensis israelensis* and *Bacillus sphaericus*) have been employed [6]. These insecticides have been reported to pose serious threats to the environment in killing nontarget species such as larval predators, bioaccumulation, hampering biodiversity, and environmental pollution [7]. Continuous application of these insecticides results in control failures, incidences of resistance, and disease resurgence owing mainly to the development of resistance in the vectors [8].

Plants contain an untapped reservoir of phytochemicals that can be used directly or as templates for synthetic pesticides. Phytochemicals have been reported to have larvicidal, pupicidal, and adulticidal activities, most being repellants, ovipositional deterrents, and antifeedants against both agricultural pests and medically important insect species [9]. Plant extracts and essential oils obtained from various plants have demonstrated promising larvicidal activities against mosquito vectors [10].

A nanoparticle is the most fundamental component in the fabrication of a nanostructure. Nanometer-sized metallic particles show unique and considerably changed physical, chemical, and biological properties compared to their macroscaled counterparts, due to their high surface-to-volume ratio [11] and various methods of making nanoparticles using plant extracts have been reported [12]. Silver nanoparticles (AgNPs) have important applications due to their very small size and large surface-to-volume ratio. They have found useful applications in various aspects of human life. Preparation of AgNPs using biological methods is constantly being exploited. Biosynthesis of AgNPs using extracts of neem (*Azadirachta indica*) [13], *Citrus limon* [14, 17], pineapple leaf [15], *Thevetia peruviana* [16], *Piper nigrum* [18], and *Ocimum sanctum* [19] has been reported. Also different matrices have been used in synthesizing AgNPs [20, 21]. Recently, our group studied the growth processes and kinetics of AgNPs synthesized using diverse plants [22].

Different researchers have reported the larvicidal activity of AgNPs synthesized using different plant extracts such as *Beauveria bassiana* [23], *Isaria fumosorosea* (Ifr) [24], *Sida acuta* [6], *Leucas aspera* [25], *Feronia elephantum* [26], *Azadirachta indica* (Neem) [27], *Morinda tinctoria* [28], *Agave sisalana* [29], *Eclipta prostrata* [30], *Ficus racemosa* [31], *Parthenium hysterophorus* [32], *Pithecellobium dulce* [33], *Couroupita guianensis* [34], *Delphinium denudatum* [35], *Agaricus bisporus* [36], *Solanum nigrum* L. [37], *Cocos nucifera* coir [38] and *Sterculia foetida* L. [39] against different stages of larvae of *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti*.

Cassia hirsuta (stinking *Cassia*) belongs to the family Caesalpiniaceae. It is a woody annual herb or undershrub herb native to Africa [40]. The plant grows as a weed in field crops, waste places, roadsides, and bush regrowths. Different parts of this plant have been used for stomach ache, dysentery, abscesses, rheumatism, hematuria, fever, and other

diseases. The seed of *C. hirsuta* contains a water-soluble gum, though not in commercial quantities, and it also contains bioanthraquinone which may prove medicinally important [41]. The petals of *C. hirsuta* were reported to contain cardiac glycosides [41] while the presence of tannins [42], phlobatanins [43], alkaloids, glycosides, anthraquinone glycosides, steroids, and flavonoids has been reported in the leaf extract [44]. The antioxidant, antibacterial, and cytotoxic activities of *C. hirsuta* leaf extract have been investigated [45] and high quantities of carbohydrate and proteins have been reported [43]. However, there have been no reports on the biosynthesis of AgNPs using *C. hirsuta*. The present study therefore aims at biosynthesizing AgNPs using *C. hirsuta* aqueous leaf extract and applying the synthesized nanoparticles in the control of *Culex quinquefasciatus*.

2. Materials and Methods

2.1. Collection of Plant Material. Fresh leaves of *C. hirsuta* were collected from Tanke area, University of Ilorin Road, and the taxonomic identification was done at the Herbarium, Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen (UIH 001/1135) was housed in the herbarium.

2.2. Collection of Mosquito Larvae. First- and second-instar larvae of *Culex quinquefasciatus* were collected from stagnant water within Ilorin metropolis, North Central Nigeria, and identified at the Entomology Unit, Department of Zoology, University of Ilorin, Nigeria. The larvae were kept in plastic trays containing tap water. They were maintained and reared in the laboratory to late III or early IV instars with dog biscuits and yeast powder in a 3 : 1 ratio [6, 46].

2.3. Preparation of Plant Extracts. The leaves of *C. hirsuta* were washed thoroughly with double distilled water (DDW) and cut into small pieces. Approximately 5 g of the leaves was then boiled with 100 mL of DDW for 10 minutes and filtered through a Whatman number 1 filter paper. The filtrate was then stored at 4°C until further use.

2.3.1. Synthesis of Silver Nanoparticles (AgNPs). In a typical reaction procedure, the aqueous extract was added to 1 mM of silver nitrate solution in 1 : 4 volume ratio. The mixture was allowed to incubate at 30°C for 30 min. The solution changed to a reddish-brown color, indicating the formation of AgNPs [16].

2.3.2. Optimization of the Reaction Conditions. Temperature, time, volume, and concentration were optimized by measuring the electronic absorption spectra for each condition. Optimum reaction volume was determined by varying the volume ratio of *C. hirsuta* aqueous leaf extract to AgNO₃ solution. The optimization of concentration was carried out by varying AgNO₃ concentration from 1 to 5 mM. Temperature optimization was conducted by incubating the reaction mixture at three different temperatures 30, 50, and 80°C.

The reaction was carried out at different intervals (5, 10, 15, 20, 30, and 45 min) to determine the optimum time.

2.3.3. Separation and Purification of Silver Nanoparticles (AgNPs). The AgNPs synthesized using *C. hirsuta* (CAgNPs) were repeatedly centrifuged at 15,000 rpm for ten minutes. The centrifugation was repeated three times. This was done to ensure that any adsorbed substances on the surface of the AgNPs were removed.

2.3.4. Characterization of CAgNPs. The synthesis of CAgNPs was monitored by UV-Vis spectroscopy. The wavelength ranged between 200 and 900 nm using the T60 UV-Visible spectrophotometer at a resolution of 1 nm. The FTIR spectra of CAgNPs and the leaf extract were recorded using Shimadzu (8400S) Fourier Transform Infrared at a resolution of 4 cm^{-1} in KBr pellets.

The crystallinity of the CAgNPs was examined using an X-ray diffractometer with monochromatic $\text{CuK } \alpha$ radiation ($\theta = 1.5406\text{ \AA}$) operating at a voltage of 40 kV and a current of 30 mA at room temperature. The intensity (AU) data for the AgNPs was collected over a 2θ range of 20° – 90° . A scanning electron microscope (SEM) equipped with an EDX attachment and a transmission electron microscope (TEM) were used to characterize the nanoparticles surface morphology and measure the size. TEM measurements were performed on a JEOL TEM 1010 transmission electron microscope at 200 kV. The TEM images were processed using ImageJ 1.42q software to obtain the particle size. Carl Zeiss ultraplus field emission scanning electron microscope (FESEM) was used at 5 kV accelerating voltage. Samples were placed on aluminum stabs using carbon tape.

2.3.5. Thermogravimetric Analysis. Thermal stability and surface weight loss of CAgNPs were analyzed by SDT Q600 V20.9 Build 20, Universal V4.7A TA Instruments, under a nitrogen gas flow of 100.0 mL min^{-1} and heating rate of $10^\circ\text{C min}^{-1}$ to 1000°C .

2.3.6. Mosquito Larvicidal Bioassay. One gram of aqueous leaf extract was first dissolved in 100 mL of DDW (stock solution). From the stock solution, a 100 mg L^{-1} solution was prepared with dechlorinated tap water for the bioassay. The larvicidal activity was assessed by the procedure of WHO [47] with some modifications [48]. Toxicity testing was performed by placing 20 late third- and fourth-instar larvae mosquito larvae into 200 mL of sterilized DDW with AgNPs in a 250 mL beaker (Borosil) at a temperature of 25°C and 16:8 h light/dark cycle. Approximately 100 mg of synthesized AgNPs was dissolved in 1 L of Milli-Q water (stock solution). From the stock solution, the nanoparticle solutions were diluted using Milli-Q water as a solvent according to the desired concentrations (10, 7.5, 5, and 2.5 mg L^{-1}). Each test included a control group (silver nitrate and distilled water) with five replicates for each individual concentration. Mortality was assessed after 24 h and 48 h to determine the acute toxicities. To avoid settling of particles especially at higher doses, all

FIGURE 1: Dose response of CAgNPs against the exposed late third- and fourth-instar larva of *Culex quinquefasciatus*.

treatment solutions were sonicated for an additional 5 min prior to addition of the mosquito larvae.

2.3.7. Dose Response Bioassay. Different concentrations (50, 40, 30, 20, and 10 mg L^{-1}) of CAgNPs were evaluated for the bioassay. The numbers of dead larvae were counted after 24 h and 48 h of exposure, and the percent mortality was reported from the average of five replicates.

Control mortality was corrected by using Abbott's formula, and percentage mortality is calculated as follows:

$$\text{Percentage Mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of larvae introduced}} \times 100. \quad (1)$$

2.4. Data Analysis. The average larval mortality data were subjected to probit analysis for calculating LC_{50} , LC_{90} , and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using BioStat computer software programs version 3.2. Results with $p < 0.05$ were considered to be statistically significant.

3. Results and Discussion

3.1. Larvicidal Activity of CAgNPs. CAgNPs showed >95% cidal activity against all the exposed late third- and fourth-instar larva of *Culex quinquefasciatus* within 48 h at concentrations higher than 10 ppm (Figure 1). At 20 and 30 ppm of CAgNPs, the percentage mortality was recorded as 96 for 24 h and 48 h. This showed that 4% of the larvae were still active. The percentage mortality recorded for 40 and 50 ppm was 100 at 48 h. This showed complete death of all the test larvae. These results suggest that the rate of the CAgNPs entry into the larvae body is not the same and the dose response bioassay showed that concentrations less than 10 ppm are suitable for larvicidal studies.

TABLE 1: Larvicidal activity of CAgNPs against different instar of *C. quinquefasciatus*.

Concentration (ppm)	Larva instar % mortality			LC ₅₀ (ppm) (LCL–UCL)	LC ₉₀ (ppm) (LCL–UCL)	Slope	χ^2 (df = 3)
	I	II	III & IV				
Control			0.00 ± 0.00				
2.5			58.0 ± 16.43				
5.0	**		76.1 ± 13.42	4.43 (3.65–5.16)	8.37 (7.84–8.90)	4.629	1.76
7.5		**	84.3 ± 11.40				
10.0			94.5 ± 8.94				

**100% mortality; LC₅₀ lethal concentration that kills 50% of the exposed larvae; LC₉₀ lethal concentration that kills 90% of the exposed larvae; percent cumulative mortality values are mean ± SD.

The larvae of *Culex quinquefasciatus* were highly susceptible to CAgNPs. Larvicidal studies were done using concentrations ranging from 2.5 ppm to 10 ppm of CAgNPs (Table 1). Mortality of larvae increased with increasing concentration. The LC₅₀ and LC₉₀ values after 24 h exposure were 4.43 ppm and 8.37 ppm, respectively (Table 1). Phenolic phytochemicals, saponins, flavonoids, and terpenoids which are also present in the *C. hirsuta* leaf extract have been reported to possess various biological activities such as insecticidal, mosquito larvicidal, ovicidal, and cercaricidal activity. We suggest that these compounds could have enhanced the efficacy of CAgNPs in causing mortality at low concentrations [30]. However, the aqueous leaf extract of *C. hirsuta* did not show any larvicidal effect. No mortality was also observed in any of the controls. The chi-square value (χ^2) was significant at the $p \leq 0.05$ level. AgNPs synthesized by green methods are environmentally benign. They have been shown to be effective as larvicidal agents, though the exact mechanism by which larva mortality occurs is unknown. It has been suggested that AgNPs easily penetrate into the mosquito larval body and bind to sulfur-containing proteins or to phosphate groups of DNA. This leads to the denaturing of some cell organelles, therefore resulting in decreased membrane permeability with loss of cellular functions and finally cell death [39].

Raman et al. [33] reported on the larvicidal activity of AgNPs biosynthesized using *Pithecellobium dulce*. These nanoparticles showed effective larvicidal activity against *Culex quinquefasciatus* (LC₅₀ = 21.56 mg L⁻¹ and $r^2 = 0.995$) due to high surface-to-volume ratio. Veerakumar et al. [6] synthesized AgNPs from *Sida acuta* leaves. Mosquito larvicidal activity of synthesized AgNPs against the vector mosquitoes of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* had the following LC₅₀ and LC₉₀ values: 21.92 and 41.07 $\mu\text{g mL}^{-1}$; 23.96 and 44.05 $\mu\text{g mL}^{-1}$; 26.13 and 47.52 $\mu\text{g mL}^{-1}$, respectively. Rawani et al. [37] reported that AgNPs synthesized by aqueous extracts of *Solanum nigrum* L. showed LC₅₀ values of 1.33, 1.59, and 1.56 ppm and LC₉₀ values of 3.97, 7.31, and 4.76 ppm for dry leaves, fresh leaves, and berries, respectively against *Anopheles stephensi*. The authors suggested that, after AgNPs reached the larvae midgut epithelial membrane, enzymes were inactivated and generated peroxides lead to cell death.

Rajasekharreddy and Rani [39] synthesized AgNPs using *Sterculia foetida* L. seed extracts and showed potential

mosquito larvicidal activity against *Aedes aegypti* (L.), *Anopheles stephensi* Liston, and *Culex quinquefasciatus* Say. These AgNPs also act as promising agents in cancer therapy. Kumar et al. [28] investigated the larvicidal potential of the larvae of *Culex quinquefasciatus* against third-instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). The value of the AgNPs at 50% lethal concentration (LC₅₀) = 1.442 ppm against *C. quinquefasciatus*. Soni and Prakash [27] synthesized AgNPs using aqueous extracts of leaves and bark of *A. indica* (Neem). The synthesized AgNPs were tested as larvicides, pupicides, and adulticides against the malaria vector *Anopheles stephensi* and filariasis vector *Culex quinquefasciatus*. The first- and the second-instar larvae of *C. quinquefasciatus* showed a mortality rate of 100% after 30 min of exposure. The pupicidal activity against *C. quinquefasciatus* was recorded as LC₅₀ of 4 ppm, LC₉₀ of 11 ppm, and LC₉₉ of 13 ppm after 3 h of exposure. In the case of adult mosquitoes, LC₅₀ of 1.06 $\mu\text{L cm}^{-2}$, LC₉₀ of 2.13 $\mu\text{L cm}^{-2}$, and LC₉₉ of 2.4 $\mu\text{L cm}^{-2}$ were obtained after 4 h of exposure. Vimala et al. [34] synthesized AgNPs using *Couroupita guianensis*. Extensive mortality rate for leaf synthesized AgNPs was reported with LC₅₀ of 2.1 ppm and LC₉₀ of 5.59 ppm and flower synthesized AgNPs with LC₅₀ of 2.09 ppm and LC₉₀ of 5.7 ppm.

3.2. UV-Visible Study. The aqueous leaf extract of *C. hirsuta* reduced Ag⁺ to Ag⁰. This was confirmed by the gradual color change of the final mixture from light yellow to yellowish brown (Figure 2(a) inset) indicating the formation of AgNPs [15]. Phytochemicals like tannins, alkaloids, glycosides, and flavonoids in the leaf extract [43, 45] are responsible for the reduction and stabilization of Ag⁺ to Ag⁰ nanoparticles. The surface plasmon resonance (SPR) band was observed at 440 nm for 30°C and 420 nm for both 50°C and 80°C, respectively. The size, shape, state of aggregation, morphology, composition, and the dielectric medium of the nanoparticles affect the SPR. Absorption of light at wavelength of 400–440 nm is due to collective oscillation of surface electrons in AgNPs [49].

3.3. Optimization Studies

3.3.1. Temperature. The electronic spectra of CAgNPs synthesized at 30°C, 50°C, and 80°C, respectively, for different time intervals were shown in Figures 2(a)–2(c). The SPR

FIGURE 2: The UV-visible spectra of CAgNPs synthesized at (a) 30°C, (b) 50°C, and (c) 80°C. Inset: gradual color change at 30°C for (A) 5 min, (B) 10 min, (C) 15 min, (D) 20 min, (E) 30 min, and (F) 45 min.

observed at 50 and 80°C (420 nm) are at shorter wavelengths, not sharp, and distinct in comparison with the SPR at 30°C (440 nm). This may be due to the decreased size and/or anisotropy degree of the AgNPs [49]. The increase of 20 nm in the SPR at 30°C showed an increase in particle size [50]. For all the temperatures used, an increase in reaction time resulted in an increase in absorbance values. This positive increment in absorption intensity as a function of time signifies enhanced reduction of Ag^+ to form Ag^0 nanoparticles. The SPR band overlapped after 15 min reaction time for all the temperatures used. This indicates rapid formation of CAgNPs. Therefore, the reaction temperature and time were optimized to 30°C and 30 min, respectively.

3.3.2. Volume Ratio. The result of the volume optimization studies ranging 4:1, 3:2, 2:3, and 1:4 of 1 mM AgNO_3 to *C. hirsuta* aqueous leaf extract, respectively, is presented in Figure 3(a). The SPR at 440 nm was maintained when one part of the aqueous leaf extract of *C. hirsuta* was mixed with four parts of AgNO_3 solution (1:4). However, a hyperchromic shift was observed when the volume 2:3 of 1 mM AgNO_3 to *C. hirsuta* aqueous leaf extract was reacted for 30 min. The SPR band at 440 nm disappeared when four parts of aqueous leaf extract of *C. hirsuta* were reacted with one part of AgNO_3 solution (4:1). This suggests that at volume, 4:1 of the extract to AgNO_3 solution, the reducing and stabilizing agents in the extract could not interact very well with the Ag^+ ions in the reaction mixture. Therefore,

FIGURE 3: The UV-visible spectra of CAgNPs synthesized at 30°C using (a) different volume ratios and (b) varying concentrations.

the optimum reaction volume ratio used throughout this experiment was 1:4.

3.3.3. Concentration Variation. The UV-vis absorption spectra of CAgNPs recorded at varying concentrations (1 mM–5 mM) are presented in Figure 3(a). The reaction was done at optimum reaction conditions (1:4 for 30 min at 30°C). The AgNO_3 solutions (1 mM–4 mM) showed the same SPR band and overlapping absorption intensity, while 5 mM exhibited a hyperchromic shift at 440 nm. This suggests that, at higher concentrations of AgNO_3 , there is also an increase in the number of AgNPs formed [51].

3.4. FTIR Study. Figure 4(a) showed the FTIR spectra of *C. hirsuta* aqueous leaf extract and CAgNPs, respectively. Sharp C=O stretching (1627 cm^{-1}), weak O-C stretching (2360 cm^{-1}), and strong, intense O-H stretching peaks of alcohols or phenols were observed in the FTIR spectrum of the extract. The appearance of weak band at 2916 cm^{-1} and 2845 cm^{-1} in the extract spectrum can be ascribed to the C-H stretching of the aliphatic $-\text{CH}_2$ moiety. The strong, intense peak at 3455 cm^{-1} is ascribed to the O-H stretch of alcohols or phenols [52]. The absorption bands at 1050 cm^{-1} , 1230 cm^{-1} , and 1384 cm^{-1} can be assigned to the C-O stretching vibrations in alcohols, phenols, ethers, esters, and carboxylic acids [53]. These are functional groups that are present in the extract [42–44]. The band at 2916 cm^{-1} and 2845 cm^{-1} disappeared in the FTIR spectrum of CAgNPs. The absorption peak at 3455 cm^{-1} in the extract became sharp and was shifted to 3464 cm^{-1} . Also C=O stretching at 1627 cm^{-1} in the extract was shifted to 1637 cm^{-1} in the spectrum of CAgNPs. It can be deduced from these that the carbonyl and hydroxyl groups present in tannins,

flavonoids, alkaloids, steroids, and glycosides are the main groups responsible for reducing Ag^+ to Ag^0 and capping the CAgNPs.

3.5. Elemental Composition and Morphological Studies. The SEM image (Figure 4(b)) shows that the CAgNPs are small, of varying sizes and aggregates. The purity and elemental composition of CAgNPs were observed by energy dispersive X-ray spectroscopy (EDX). The EDX of CAgNPs (Figure 4(c)) shows that Ag makes up 81% of the sample, followed by Cl (11.46%), C (6.29%), and lastly O (1.26%), which may originate from the biomolecules attached to the surface of CAgNPs. The highest count at 3 keV is characteristic of AgNPs. This shows that the synthesis procedure is efficient in producing AgNPs using *C. hirsuta* leaf. The inset of Figure 4(c) shows the percentage weight of the elements in AgNPs.

The TEM analysis showed that CAgNPs formed at optimum reaction conditions were of spherical morphology, with few larger anisotropic nanoparticles being observed (Figures 5(a) and 5(b)). The particle size ranged from 4.1 to 11.9 nm. The particle size distribution as shown in the plot gave the average size as $6.9 \pm 0.1\text{ nm}$ (Figure 5(c)). Selected Area Electron Diffraction (SAED) showed that CAgNPs were crystalline with the spots arising from Bragg reflection (Figure 5(d)).

3.6. XRD Analysis. The crystallinity of CAgNPs was confirmed by X-ray diffraction (XRD) patterns. The spectrum showed that diffraction peaks at 38.5° , 44.5° , 65° , 77.8° , and 82° are indexed to (1 1 1), (2 0 0), (2 1 1), (2 2 0), and (2 2 2) planes of a face center cubic (fcc) lattice of silver crystal. The diffraction peaks were matched with the data of JCPDS number 01-087-0717 [54]. The peak 111 was observed

FIGURE 4: (a) FTIR spectra of extract and CAgNPs (b) SEM images of CAgNPs (c) EDX spectra of CAgNPs. Inset: percentage weight of the elements in CAgNPs.

as the major orientation (Figure 6). This is similar to reports from other researchers [55, 56]. Other peaks (*) suggest the crystallization of bioorganic phases in the plant extract [57–59].

3.7. Thermogravimetric Analysis. Figure 7 showed the DSC-TGA of CAgNPs synthesized at the optimum reaction conditions. The percentage weight loss was shown from 30°C to 1000°C. The initial weight loss of about 2% at the temperature of 70°C was due to loss of water molecules from CAgNPs. The weight loss as shown by the TGA graph proceeded in two steps. The first occurred between 70 and 175°C, while further degradation (about 18%) was observed between 170 and 400°C. The degradation which commenced at 170°C was suggested to be the decomposition of the bioorganic moiety from CAgNPs. Thermal degradation of CAgNPs was not observed from 400°C to 1000°C. This showed the stability of metallic silver. Also the analysis of DSC curve

showed an endothermic peak at 65°C. This matches with the denaturation enthalpy of CAgNPs at 70°C observed in TGA curve. This trend was also reported by Mata et al. [60].

4. Conclusion

Successful synthesis of AgNPs using aqueous extracts of *C. hirsuta* leaves has been carried out and its larvicidal potentials are studied. Crystalline spherical CAgNPs of size 6.9 ± 0.1 nm were reduced and stabilized by the phytochemical constituents in the extract. Carbonyl and hydroxyl groups were identified by FTIR to participate in the synthesis of AgNPs. CAgNPs showed good mosquito larvicidal potential against *Culex quinquefasciatus*. The AgNPs were also synthesized at different temperatures. The low cost, energy saving, easy scale-up, and environmental benign method of synthesis was further ascertained by successful synthesis at 30°C. Therefore,

FIGURE 5: TEM images of CAgNPs at (a) $\times 250$ K M, (b) $\times 400$ K M, (c) particle size distribution, and (d) SAED pattern of CAgNPs.

FIGURE 6: XRD spectrum of CAgNPs.

FIGURE 7: DSC-TGA graph of CAgNPs.

CAgNPs are promising candidates in controlling the menace of mosquitoes in our environment.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors wish to acknowledge the Vice Chancellor of Federal University Lafia, Professor Ekanem Ikpi Braide and Dr. A. O. Oduola of the Department of Zoology, University of Ilorin, Ilorin, Nigeria, for their support and help during the course of this research work.

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