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## Ecofriendly production of silver nanoparticles from the seeds of *Carica papaya* and its larvicidal and antibacterial efficacy against some selected bacterial pathogens

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## Ecofriendly production of silver nanoparticles from the seeds of *Carica papaya* and its larvicidal and antibacterial efficacy against some selected bacterial pathogens

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### Abstract

*Carica papaya* seed extract (CPE) was used in the synthesis of silver nanoparticles (AgNPs) in this study. For the characterization of biosynthesized particles, UV-vis spectroscopy, FTIR, FESEM and EDX were used. Antimicrobial and larvicidal efficacies of the synthesized AgNPs were assessed in the fight against certain pathogens and the *Aedes aegypti* 4th instar larvae. The absorption spectrum of AgNPs peaked at 433 nm with a broad peak of 3000 to 3800  $\text{cm}^{-1}$  for different functional groups as presented in the FTIR analysis. A FESEM evaluation revealed a number of spherical particle structures with an average of 20-69 nm. With zones of inhibition between 10-24 mm, the AgNPs synthesized inhibited selected microorganisms. After 12 h of exposure, the nanoparticles had  $\text{LC}_{50}$  and  $\text{LC}_{90}$  lethal concentration on the *Aedes aegypti* larva at 14.56 and 33.89  $\mu\text{g/ml}$  respectively. This study demonstrates possibility of using *Carica papaya* seeds in AgNPs synthesis.

**Keywords:** Antibacterial, *Carica papaya*, dengue fever, larvicidal, pathogens, silver nanoparticles



## 1 Introduction

The development of microbial resistance to antimicrobial agents is one of the biggest challenges facing bio-scientists. Zika and dengue fever both transmitted by *Aedes aegypti* mosquito are another major public health problem that bio-scientists are looking to tackle. One way to control these mosquito-borne diseases is to interfere with the transmission of diseases through either the eradication of mosquitoes, the preservation of mosquitoes against bitten persons or through a high-scale mortality in mosquito larvae at vector breeding sites [1]. The application of chemical larvicides is detrimental to the environment because they are not biodegradable and therefore can persist for a long time in the environment [2]. In addition, synthetic larvicidal also disrupt biological frameworks of natural control which at times promote resistance [3]. Scientists are therefore looking for alternative natural products that can serve as both larvicidal and antimicrobial agents.

Silver has been known for a long time to prevent microbial contamination. It protects against quite a number of microorganisms such as *Escherichia coli* and *Pseudomonas aeruginosa* [4]. However, the metal has received much attention recently and is being used in the synthesis of silver nanoparticles. *Azadirachta indica* leaf extract used in silver nanoparticles synthesis (AgNPs) was reported by Benakashani *et al.* [5]. Numerous researchers in silver nanoparticles synthesis with remarkable biomedical applications also used several other species including *Cola nitida*, *Theobroma cacao*, *Buchholzia coriacea* and *Synsepalum dulcificum* [6-14].

In biomedical research, the different applications for certain parts of the *Carica papaya* plant have been used a number of times. Okeniyi *et al.* [15] documented the anti-moebic and antihelmintic potentials in *Carica papaya*. Bamisaye *et al.* [16] also reported on the plant's ethnobotanical perspectives. The antimicrobial efficacy of its root against certain infectious microorganisms was also tested by Tiwari *et al.* [17]. In a previous paper, the flavonoid content, total phenolic content and total antioxidant activity of the various plant parts of the papaya tree were determined and compared [18].

Several portions of the *Carica papaya* plant have also been utilised for green synthesis of nanoparticles. Jain *et al.* [19] reported the usage of *Carica* fruit extract in AgNPs synthesis. Its antimicrobial efficacy against some microorganisms such as *Escherichia coli* and *Pseudomonas aeruginosa* were likewise reported [19]. In addition, Kokila and others in 2013 also reported the usage of the AgNPs synthesized from its peel extract as antioxidant and antimicrobial agents [20]. Mahanty *et al.* [21] evaluated the possible application of silver nanoparticles in aquaculture as alternatives to antibiotics. Banala *et al.* [22] also evaluated the

bactericidal activity of silver nanoparticles synthesized from the leaf extract of this plant against human pathogenic strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Microbacterium luteus*, *Klebsiella pneumoniae* and *E. coli*. In the synthesis of AgNPs, Mude *et al.* [23] also used the callus extract to synthesize AgNPs, while Sankar *et al.* [24] took further steps in the synthesis and application of colloidal copper oxide in photocatalytic degradation with the extracts from this plant. It is worthy of note in the field of nanotechnology that nanoparticles from the seeds of *Carica papaya* have not been synthesized or investigated for its biological or antimicrobial properties. Therefore, the study discussed here aims to analyze and apply *Carica papaya* seed as antimicrobial, larvicidal and bio-reducing agents in the green synthesis of AgNPs.

## **2 Materials and Methods**

### **2.1 Sample collection**

Fresh *Carica papaya* was purchased from Ilishan-Remo market in Ogun state and was stored in the Babcock University Microbiology Laboratory. The fruit was cut open and the seeds were removed and dried for 5 days ( $30 \pm 2^\circ \text{C}$ ) under shade. The dried seeds were pulverised and stored in air-tight bottles at room temperature for further use in water/air resistant bottles. For antimicrobial studies, *Staphylococcus aureus* was the selected Gram positive organism used, while *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* served as the selected Gram negative organisms. These bacterial strains were collected from the Teaching Hospital of Ladoke Akintola University Ogbomoso, Nigeria.

### **2.2 Collection and Identification of Mosquito Larva**

The mosquito larvae were collected using a net, and kept in sealed containers from polluted body of water in Babcock University, Nigeria. In this study, the target mosquito larvae were the 4th instar larva *Aedes aegypti* mosquito. Identified *Aedes aegypti* mosquito larvae (identified by a medical entomologist) were isolated from the other mosquito species and placed separately in a container full of water.

### **2.3 Preparation of extract**

The *Carica papaya* seed aqueous extract used for this analysis was done following the method of Lateef *et al.* [25]. One gram of the seed was soaked in 100 ml distilled water. This was accompanied by heating in the water bath for 1 h at  $60^\circ \text{C}$  temperature. The extract was filtered and centrifuged at 4000 rpm for 20 min and the resultant supernatant was obtained.

## 2.4 Biogenic synthesis of the AgNPs

The AgNPs was biogenically synthesised by introducing 1 ml of the prepared extract into 40 ml of 1 mM AgNO<sub>3</sub> and the colour change was observed. The entire reaction which was about 2 h was conducted in ambient condition [25].

## 2.5 Characterization of the AgNPs

The synthesized AgNPs were characterized by a UV-visible spectrophotometer (Cecil USA) that measured its absorbance spectrum at 190-1100 nm. FTIR spectroscopy identified biomolecules participating in the green synthesis. The FTIR model 8400S (Shimadzu Japan) developed vibrational frequencies in NPs from FTIR spectrum using KBr process. To assess the identity and ratio of elemental particles, EDX analysis (Silicon-Drift Detector X-Max daN Oxford Instruments UK) was carried out. FESEM (Lyra 3 Tescan Czech Republic) was used to examine the presence, size and structural morphology of synthesized silver nanoparticles. Image J was used to analyze the image attributes [26].

## 2.6 Antimicrobial Activity

The agar well-diffusion method was used to assess the efficacy of biosynthesized *Carica papaya* extract mediated silver nanoparticles (CPE-AgNPs) against certain selected clinical isolates. An 18 h-old culture of each species grown in peptone broth was used to inoculate an already prepared Mueller Hinton agar plate. Holes were drilled using a 6 mm sterile cork borer on the inoculated plate and labelled 10, 20, 40, 60, 80 and 100 µg/ml respectively. The resulting holes were then filled with 100 µl of different concentrations from the synthesized CPE-AgNP silver nitrate (positive control) and water (negative control). The plates were incubated at 37 °C for 18 h after which the zone of inhibition was then determined [27]. The analysis was done in triplicates.

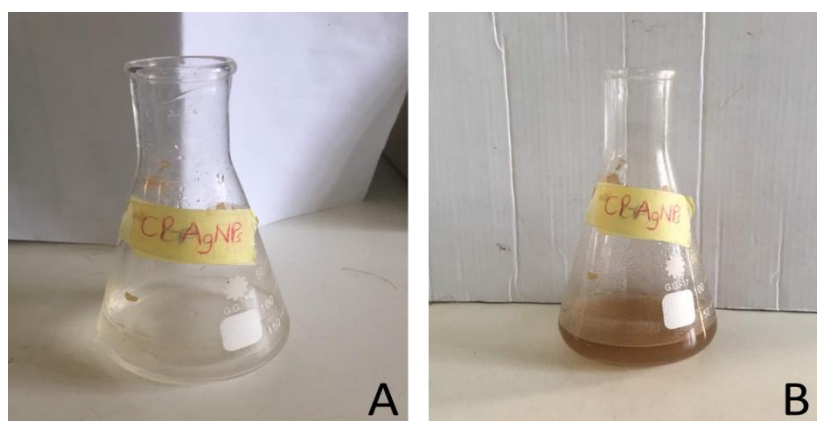
## 2.7 Larvicidal Efficacy

The WHO recommended guideline was used to test the efficacy of the AgNPs with the 4th *Aedes aegypti* instar larva [28]. A microtitre plate of 96-hole was used. The 5 holes of 20, 40, 60, 80 and 100 µg / ml respectively were loaded with five larvae. In each respective hollow, 300 µl of each AgNP concentration was then dispensed as labelled. A control experiment was conducted in which the larvae were exposed to sterile distilled water. Triplicate tests were performed and after 12 h of treatment readings were obtained. This was followed by statistical assessment of mortality percentage and Probit analysis for the LC<sub>50</sub> and LC<sub>90</sub> computation.

### 3 Results and Discussion

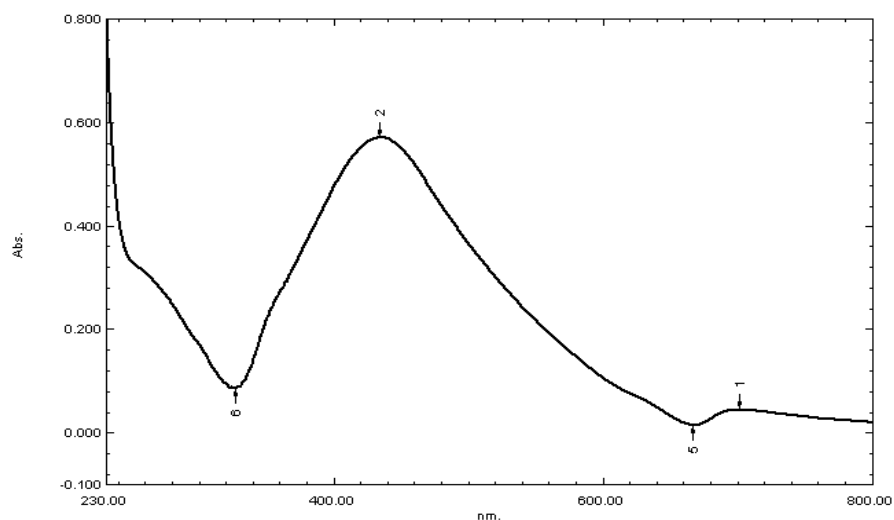
#### 3.1 Synthesis and Characterization of the Synthesized AgNPs

Upon the addition of the plant extract to the silver nitrate, the solution still remained colourless. After 10 min, a gradual change in colour was observed; initially starting faintly and in 30 min, a stable deep brown colouration was formed in the reaction vessel (Figure 1), which is indicative of the formation of AgNPs as it has been observed by several other researchers [8-12]. The colour change of the solution of metallic salt is a sign of nanoparticles development. Earlier researchers reported extensively the advances of different color shades including light yellow to darker colloidal AgNPs [4, 7-9, 29-32]. A light brown coloration was observed by Mahanty *et al.* [21] during the synthesis of silver nanoparticles from *Carica papaya* leaves. It was believed that this colour shift during the formation of silver nanoparticles from *Carica papaya* is induced by the different biomolecules present in the plant and are of responsible for the synthesis and stability of silver nanoparticles in its seed extract [8].



**Figure 1.** Synthesis of the CPE-AgNPs (a) 0 min after the addition of the plant extract to the silver nitrate (b) Formation of deep brown colouration after 30 min

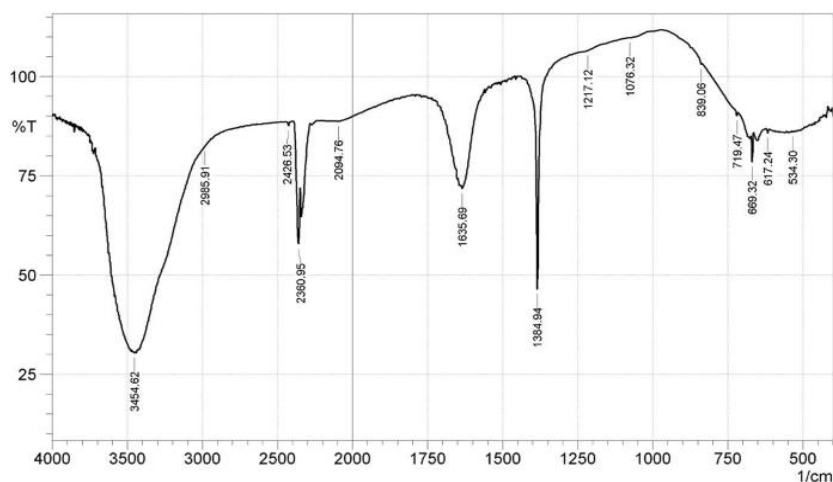
The CPE-AgNPs formed were examined with the UV-vis spectrophotometer as shown in Figure 2. The absorption range of the synthesized CPE-AgNPs displayed a peak magnitude at 433 nm. The peak is in line with previously reported investigations on silver nanoparticles [4, 8, 25, 33-35]. Jain *et al.* [19] recorded a maximum absorption of 450 nm which was due to surface plasmon excitation when using *Carica papaya* fruit extract for silver nanoparticles synthesis.



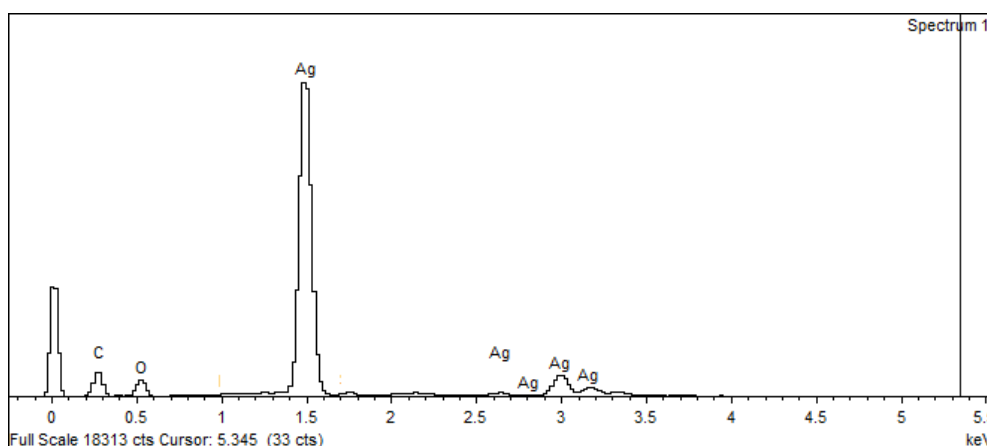
**Figure 2.** UV-vis spectra of AgNPs synthesized from *Carica papaya* seed extract

Specific functional classes on the surface of the AgNPs were shown in the FTIR study of the CPE-AgNPs (Figure 3). It has a large range from 3000 to 3800  $\text{cm}^{-1}$  defined as the ones with vibration of O-H and/or N-H correlated with n-substituted amide [36] at 2426  $\text{cm}^{-1}$  and 2360  $\text{cm}^{-1}$ , and the ones with ambient  $\text{CO}_2$  absorption [37-38]. The N-H bending of amides of proteins is reported at 1635  $\text{cm}^{-1}$ .

The EDX ranges of the *Carica papaya* controlled AgNPs is shown in Figure 4. Powerful signals at 1.5 keV and 3.0 keV were recorded from Ag atoms while weak signals at 2.7, 2.9 and 3.2 keV were noted. Figure 4 also displayed certain components such as C and O which are known as impurities from the sample. Several other scientists [39-41] reported similar findings of heavy silver signalling varying from 1.5 to 5.0 keV.

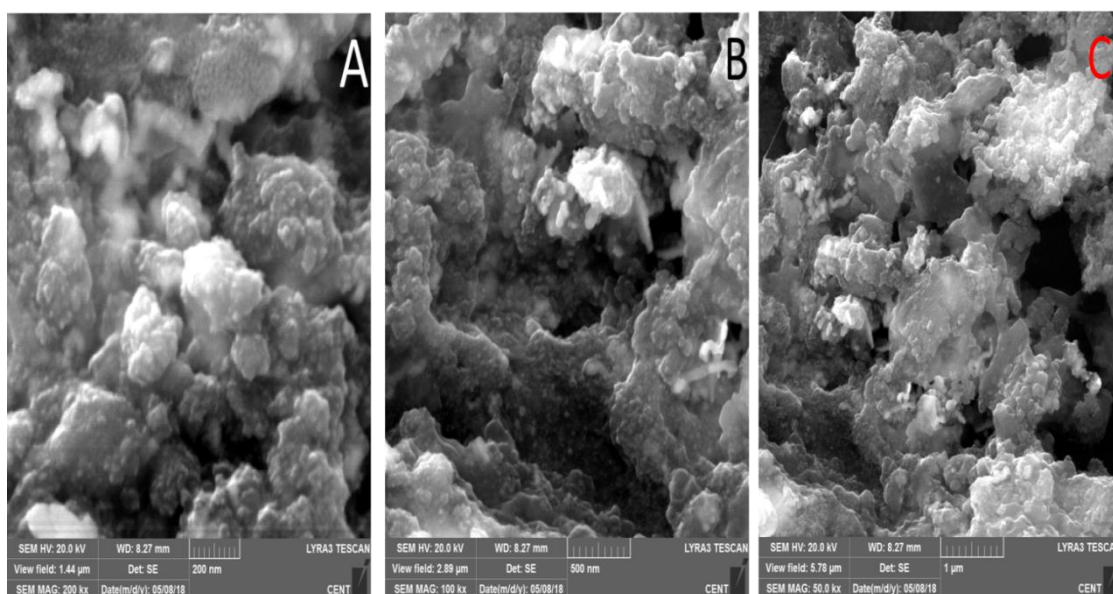


**Figure 3.** The FTIR spectrum of the synthesized AgNPs



**Figure 4.** Energy dispersive spectrum of the synthesized AgNPs

In Figure 5, FESEM analysis on CPE-AgNPs provided additional details on the size and morphology of the synthesized AgNPs. As a consequence of its high surface ratio, the particles produced formed clusters or aggregates. There are also many spherical nanoparticles from 20 to 69 nm. This picture shows a large amount. The viability of *Carica papaya* seed for the biosynthesis of silver nanoparticles in this study has been verified by similar FESEM findings from synthesized silver nanoparticles from other plants [8].



**Figure 5.** Field emission scanning electron micrograph of the synthesized AgNPs at different magnifications (a) 200kx (b) 100kx (c) 50kx

### 3.2 Antimicrobial activity

Of all the four selected pathogens tested, the synthesized AgNPs inhibited the growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*, all of which were



inhibited distinctly with zones ranging from 10-24 mm. A wide range of pathogenic organisms have been inhibited by biosynthesized AgNPs effectively [42-46]. At 100 µg of CPE-AgNPs *Staphylococcus aureus* was inhibited by 16.0 mm, while other concentrations (10-80 µg) did not have any inhibitory effect. Panacek *et al.* [47] reported similar resistance trends to AgNPs. It is proposed that flagellin present in large amounts in bacteria may cluster the silver nanoparticles and thus decreases its antibacterial effectiveness. Ultimately, the results of this study showed a significant antibacterial impact of the nano-sized silver synthesized from *Carica papaya* seed. The above details are described in both Table 1 and Figure 6.

**Table 1.** Zone of inhibition of the synthesized AgNPs against some selected pathogens

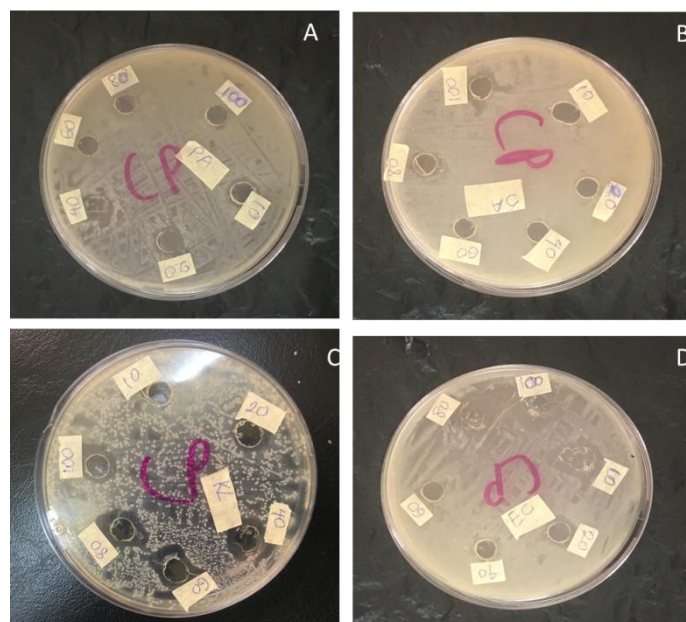
| Isolate                      | Mean Zone of Inhibition (mm) ± standard deviation (SD) |         |         |         |         |          | AgNO <sub>3</sub> | CPE | Distilled<br>H <sub>2</sub> O |
|------------------------------|--------------------------------------------------------|---------|---------|---------|---------|----------|-------------------|-----|-------------------------------|
|                              | AgNPs                                                  |         |         |         |         |          |                   |     |                               |
|                              | 10µg/ml                                                | 20µg/ml | 40µg/ml | 60µg/ml | 80µg/ml | 100µg/ml |                   |     |                               |
| <i>taphylococcus aureus</i>  | NZ                                                     | NZ      | NZ      | NZ      | NZ      | 16.0±01  | 6.2±02            | NZ  | NZ                            |
| <i>lebsiella pneumoniae</i>  | NZ                                                     | 11±02   | 17±02   | 24±02   | 20±02   | 15.0±01  | 6.4±01            | NZ  | NZ                            |
| <i>seudomonas aeruginosa</i> | 10±01                                                  | 12±02   | 13±01   | NZ      | NZ      | NZ       | 6.1±01            | NZ  | NZ                            |
| <i>scherichia coli</i>       | 13±01                                                  | 10±01   | NZ      | NZ      | 12±02   | 20.0±01  | 6.3±01            | NZ  | NZ                            |

NZ, No zone

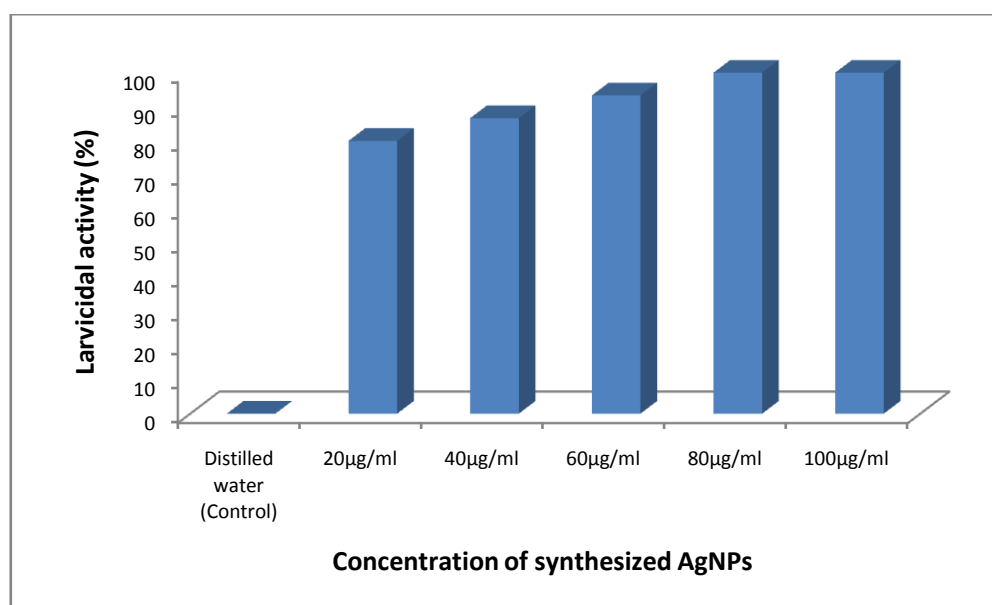
### 3.3 Larvicidal Activity

The biosynthesized AgNPs were found to be highly toxic to the 4<sup>th</sup> instar larvae *Aedes aegypti*. Mortality percentages in the 20, 40 and 60 µg/ml were found to be 80%, 86.6% and 93.3% respectively, while the 80 and 100 µg/ml showed 100 % mortality as shown in Figure 7. However, no mortality was recorded in the control group. Furthermore, the AgNPs exhibited a concentration dependent activity against mosquito larvae since the percentage mortality was observed to increase with increasing concentrations of the biosynthesized nanoparticles. The 50 % lethal concentration (LC<sub>50</sub>) of the nanoparticles was 14.54 µg/ml, while the LC<sub>90</sub> was 33.9 µg/ml. Similar results were recorded also in the previous study by Lateef *et al.* [25], where AgNPs synthesized from cell-free extract of *Bacillus safensis* exhibited larvicidal potential against Anopheline larvae. 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. CPE-AgNPs larvicidal activities were similar to

reported larvicidal activity of some AgNPs of bacterial extract origin and plants against *Aedes aegypti* larvae [48].



**Figure 6.** The antibacterial activities of the CPE-AgNPs against some clinical bacterial isolates (a) *Pseudomonas aeruginosa* (b) *Staphylococcus aureus* (c) *Klebsiella pneumoniae* (d) *Escherichia coli*



**Figure 7.** Larvicidal activity of the biosynthesized CPE-AgNPs on *Aedes* mosquito larvae

#### 4 Conclusion

This study has revealed a cheaper and eco-friendly biosynthesis of AgNPs using seeds of *Carica papaya*. It is proof that the seeds of the plant possess the biomolecules and phytochemicals necessary for the reduction and stabilization agents in the synthesis of silver nanoparticles. The CPE-AgNPs had remarkable antibacterial and larvicidal activities, making available a new agent for combat against multi-drug resistant bacteria and the Zika and Dengue fever vector. More investigation into the cytotoxicity of these biosynthesized silver nanoparticles is however required to further clarify issues on its application.

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