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# Carissa carandas L. fruit mediated in vitro synthesis of silver nanoparticles and their antioxidant and antibacterial activities

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ORIGINAL STUDY

# *Carissa Carandas* L. Fruit Mediated *In Vitro* Synthesis of Silver Nanoparticles and Their Antioxidant and Antibacterial activities

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**Abstract**

**Currently, we synthesized silver nanoparticles (AgNPs) from the aqueous fruit extract of *Carissa carandas* L. and evaluated their antioxidant and antibacterial properties. The UV−visible spectra showed a characteristic absorption peak at 412 nm. The Fourier transform infrared (FTIR) spectroscopy exhibits intense peaks at 3430.23 $cm^{-1}$ and 1625.67 $cm^{-1}$ which indicates the involvement of flavonoids and other functional groups in the biosynthesis, capping, and stabilization of AgNPs. From XRD analysis, the average size of AgNPs was estimated to be 45 nm. The biogenic AgNPs were spherical in shape and average size range between 10 and 95 nm confirmed by HR-TEM analysis. The fabricated AgNPs by fruit extract were monitored for antioxidant activity using DPPH method. The fruit extract of AgNPs (IC50 = 946.22 μg/ml) had the highest antioxidant activity against standard ascorbic acid. The antibacterial activity was done using *Staphylococcus aureus* and *Escherichia coli* bacterial strains. It was found from the results that the bacterial strain *S. aureus* had the highest antibacterial activity when compared to *E. coli* and aqueous fruit extract. Thus, the present study exhibits synergistic antioxidant and antibacterial activity which may explore in future clinical treatment.**

*Keywords:* Biosynthesis, Silver nanoparticles, Characterization, Antioxidant, Antibacterial

## 1. Introduction

Plant mediated silver nanoparticles had received more attention towards synthesis, design and manufacturing of particles ranging between 1 and 100 nm. The unique properties of nanoparticles have been exploited in a wide area of potential applications in medicine, catalytic, agriculture, food, renewable energies, and environmental remediation's [1,2]. Noble metals like silver, zinc, copper, titanium, magnesium, gold and platinum had various biomedical applications in the areas of antioxidant, antibacterial, anti-inflammatory, and anticancer activities that are well documented [3,4]. These activities are predominantly depending upon particle size and structure (atomic or molecular). The smaller size and larger surface area of AgNPs may enter easily into the living cells via cellular endocytosis and pinocytosis mechanisms resulting exhibit potential disease inhabiting activity [5].

Even though, chemical and physical syntheses of AgNPs are associated with toxic chemicals and exhibit higher toxicity during *in-vitro* synthesis. On the other hand, plant-mediated synthesis of nanoparticles has drawn much scientific attention because of low toxicity, reliable, less time consumption, cost-effeteness, and ecofriendly [6]. Thus eco-friendly procedures use green materials like microbes, algae and plants to synthesize NPs [7,8]. In addition, plants are a good source of secondary metabolites including flavonoids, tannins, alkaloids, phenolic compounds, carboxylic acids, amines, proteins, ketones, and aldehydes can act as reducing, capping and stabilizing agents in the conversation of silver ions to silver nanoparticles [2,9]. The reduction of silver ions is mainly due to the conversion of nitrate ($NO_3^-$) to nitrite ($NO_2^-$),

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electron is transferred to silver ions ($Ag^{+}$) which are then reduced to metallic silver ($Ag^{0}$) [10]. Accumulation of AgNPs can be achieved either by the intracellular or extracellular process. Recent studies have reported that intracellular accumulation of AgNPs induces destabilization of the plasma membrane, blocks bacterial respiration; depletes ATP production resulting denature cell wall of bacteria [11].

*Carissa carandas* L. (Karonda) is a deciduous evergreen shrub that belongs to the family Apocynaceae commonly known as crane berry. Traditionally, plant and their parts have been used in the treatment of cancer, scabies, anthelmintic, intestinal worms, hepatoprotective and antiscorbutic activity was reported [12]. The plant leaves are rich in various phytochemicals like flavonoids, iron and Vitamin C which possess well-known high antioxidant, antibacterial and antifungal activity against human pathogenic bacteria [13]. The leaves and bark of *C. carandas* exhibit good antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* [14]. Hence the present investigation we studied antioxidant and antibacterial activity of fabricated AgNPs from fruit extract of *C. carandas*.

## 2. Materials and methods

### 2.1. Plant materials and chemicals

Fresh fruits of *C. carandas* were collected from wild plants situated at Karnatak Akkamahadevi Women's University campus Vijayapur, Karnataka, India. All the chemicals and analytical grades were purchased from Hi Media Pvt. Ltd. Mumbai, India. The experiments were performed using Millipore double distilled water (DDW).

### 2.2. Fruit extract preparation

Healthy ripen fruits were collected from wild plants. The collected fruits were surface sterilized 2 to 3 times with running tap water followed by double distilled water to remove adhered dirt and other contamination from the fruits. The sterilized fruits were air-dried for 30 min. Thereafter, fruits (10 gm) were chopped into fine pieces and transferred to 250 ml Erlenmeyer flask containing 100 ml of DDW. The solution was boiled for 15–20 min at 60 °C. Further, the sample was allowed to cool at room temperature and the solution was filtered through Whatman filter paper No. 1. The filtrate was stored under refrigerator at 4 °C until further analysis.

### 2.3. Biofabrication of AgNPs

For green synthesis, 10 ml of aqueous fruit extract of *C. carandas* was added to 90 ml of silver nitrate solution. After 20 min the reaction mixture gradually changed its color from pale peach to maroon. Further, the reaction mixture was adjusted to pH 8, 9, and 10 with 0.01 N Hcl and 0.01 N NaoH, color change was observed from maroon to dark brown indicating the formation of AgNPs and reduction of silver ions.

### 2.4. Characterization

The stability and formation of AgNPs were monitored by UV–Visible spectral analysis (UV–1650PC-Shimadzu Europe). Before measuring the reaction mixture (1 ml) was diluted with double distilled water (2 ml) and used as a blank solution. The absorption spectrum was recorded between 200 and 800 nm with 1 nm resolution. The sample was centrifuged (Remi 8C-T-10 M) at 10,000 rpm for 15 min resulting in the formation of pellets in pelletized form. The pellets were purified thrice with 3 ml distilled water by centrifugation and dispersion process. Finally, obtained pellets were dried at 60 °C in an oven. Thereafter, purified powder of AgNPs was subjected to FTIR measurement using potassium bromide (KBr) pellets. The pellet was measured in the range between 4000 and 400 $cm^{-1}$ to identify the involvement of phytochemicals in AgNPs and fruit extract. The face-centered cubic (fcc) structure, crystalline nature, and size of the AgNPs were subjected to X-ray diffraction (XRD) spectrometer measurement scanned in the range 30–90 (2θ) degrees. The surface morphology and particle size of the AgNPs were analyzed using high-resolution transmission electron microscope (HR-TEM) modal JEOL 3010.

### 2.5. Evaluation of antioxidant assay

For determination of free radical scavenging activity of AgNPs derived from *C. carandas* fruit extract was analyzed by DPPH (1,1, dipheny 1–2 picrylhydrazyl) method [15] with slight modifications. The biogenic AgNPs prepared with different concentrations viz. 50, 100, 150, 200 and 250 μg/ml. The ascorbic acid was used as standard and fruit extract as control. Further, 0.3 mM (1 ml) DPPH was added into the different test tubes containing 0.1 mM of methanol and were thoroughly vortexed. The test tubes containing solution were incubated for 20 min in a dark place. For control, AgNPs are replaced by fruit extract and ascorbic acid as standard. The

solution of sample (DPPH) was monitored by measuring absorbance at 517 nm using UV–Visible spectroscopy and methanol as blank.

### 2.6. Assessment of antibacterial activity

In vitro antibacterial activity of AgNPs synthesized from fruit extract of *C. carandas* was determined by well diffusion method against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains. Different concentrations of AgNPs viz. 50, 100, 200, and 400 μg/ml and fruit extract was used as control (200 μg/ml). The desired quantity of AgNPs solutions was filled in a well. Thereafter incubate the culture in an incubator at 37 °C for 18 h for bacterial growth. After incubation, the zone of inhibition was observed from each well and measured in mili meter (mm).

### 2.7. Stastical analysis

The statistical analysis was determined using SPSS software 20 (IBM Armonk, NY, USA). The data of the samples were performed by one-way ANOVA (Mean ± SE) and made the comparison with the standard reference. The results were found to be statistically significant at $p<0.05$.

## 3. Results and discussion

### 3.1. UV–visible spectral analysis

The reduction and capping of AgNPs derived from fruit extract of *C. carandas* determined by UV–Visible spectroscopy (Fig. 1). The color of the reaction mixture proved the formation of an absorption prominent peak is due to the excitation of plasmon resonance [16]. The obtained different prominent peaks of AgNPs in the visible ranges 412 nm, 426 nm, and 410 nm in pH 8, 9, and 10 respectively. The SPR red shift represents the highest intense peak of AgNPs ($\lambda_{max}$ = 426 nm) at pH 9. A similar result confirms the findings of Umashankari et al. [17], using *Rhizophora mucronata* (leaf extract of mangrove plant). The single intense peak of SPR exhibits spherical-shaped nanoparticles, whereas two or more peaks indicated that formations of NPs are anisotropic [18].

### 3.2. FTIR spectroscopy analysis

FTIR spectroscopy was used to determine the possible involvement of phytochemical compounds in the fruit extract and AgNPs solution. The different functional groups were identified based on absorbance bands. The intense peak of AgNPs synthesized from fruit extract of *C. carandas* was shown in Fig. 2 and Table 1. The absorbance band 3430.23 $cm^{-1}$ and 2923.87 $cm^{-1}$ could be due to the stretching of N–H/O–H groups and symmetric vibrational mode of methylene. The peak at 1625.67 $cm^{-1}$ was assigned to C–N stretch vibration medium of arenes [19]. The intense peak 1416.41 $cm^{-1}$ corresponds to stretching of amide band I and bending of silver ions with carboxylate groups. The prominent band 1384.55 $cm^{-1}$ corresponds to C=C stretching of aromatic compounds which confirmed the presence of flavonoid [20]. The

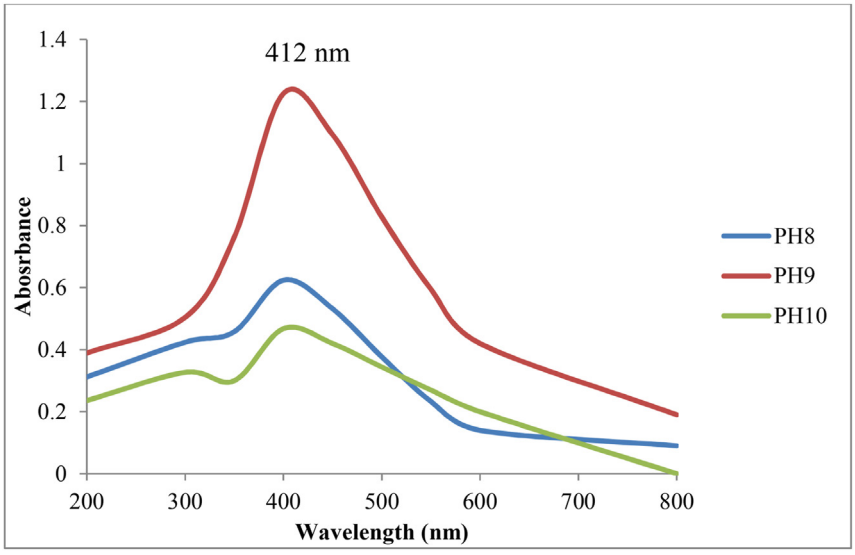


*Fig. 1. UV–Visible absorbance spectra of AgNPs fabricated from fruit mediated extract of C. carandas.*

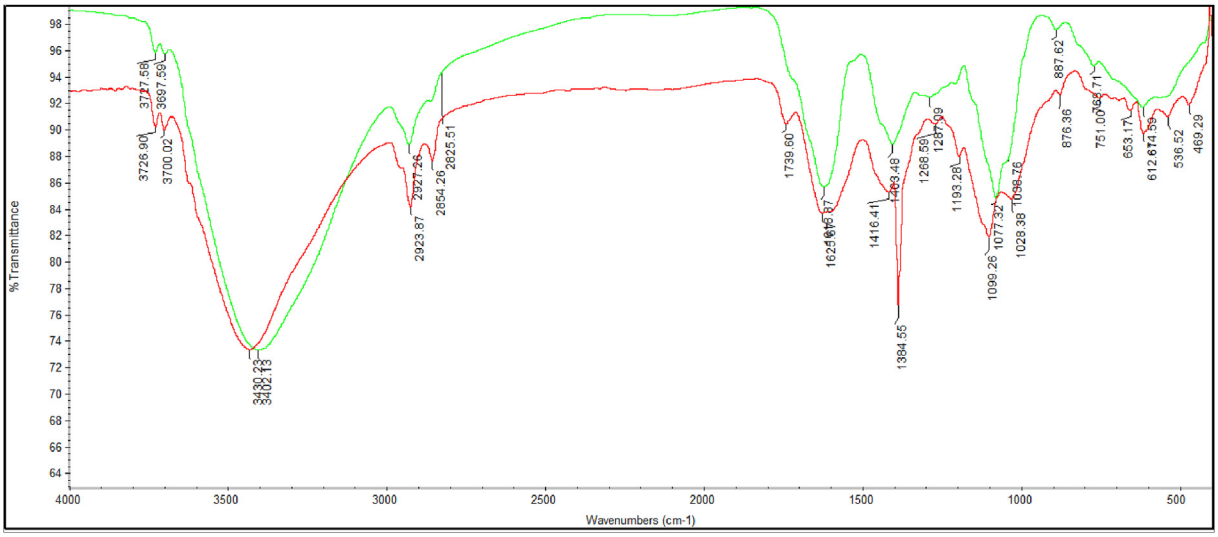


*Fig. 2. FTIR absorption peaks of fruit extract and synthesized AgNPs of C. carandas.*

*Table 1. FTIR absorption peaks aqueous fruit extract of C. carandas and synthesized AgNPs.*

| Sl. No. | Fruit extract | Silver nanoparticles | Functional groups |
|---|---|---|---|
| 1 | 3402.13 | 3430.23 | N–H/O–H stretching |
| 2 | 2927.26 | 2923.87 | Methylene symmetric vibrational mode |
| 4 | – | 1739.60 | Carbonyl stretching |
| 5 | 1618.87 | 1625.67 | Amide band I/Carbonyl stretch |
| 6 | 1403.48 | 1416.41 | Bending of silver ions with carboxylate groups |
| 7 | – | 1384.55 | -C-O- stretching of the carboxylation ions |
| 8 | 1287.09 | 1268.59 | Amide band III |
| 9 | – | 1193.28 | -C-O-C- linkages |
| 10 | 1077.32 | 1099.26 | C–N stretching vibration of the amines/Ether linkages |
| 11 | 1038.76 | 1028.38 | C–N stretching of aliphatic amines |
| 12 | 768.71 | 751.00 | Amide band V bending of peptide linkages of proteins |
| 13 | – | 653.17 | Assigned out of plane bending vibrations of substituted ethylene system to CH |

1193.28 $cm^{-1}$ is assigned to the –C-O- stretching of the carboxylation ions and -C-O-C- linkages. The peak at 1099.26 $cm^{-1}$ and 1028.38 $cm^{-1}$ corresponds to C–N stretching vibration of the amines/Ether linkages and C–N stretching of aliphatic amines. The intense peaks at 751.00 $cm^{-1}$ and 653.17 $cm^{-1}$ arises due to bending of peptide linkages of proteins and bending vibrations of substituted ethylene system to CH. These biomolecules are responsible for reducing, stabilizing and capping of AgNPs during synthesis.

### 3.3. XRD analysis

Crystalline size and structure of the bio-synthesized AgNPs of *C. carandas* fruit extract were carried out by X-ray diffractometer (Fig. 3). The powder of the AgNPs was taken for the 2θ range of 30–90°. The XRD analysis of AgNPs shows characteristic diffraction peaks at 2θ = 37.74 °, 44.90 °, 64.32

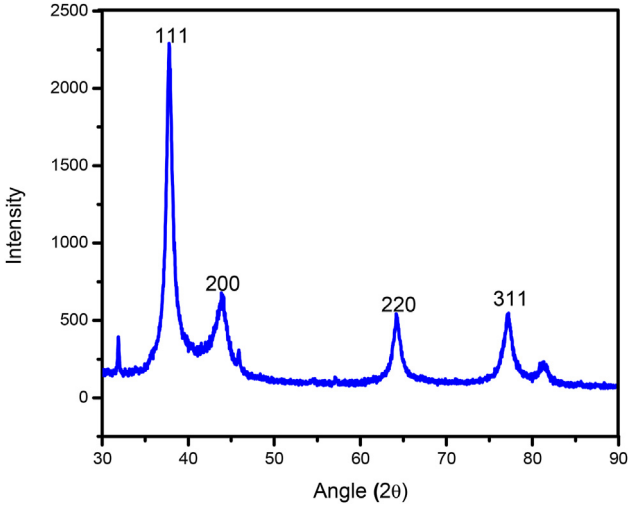


*Fig. 3. X-ray diffraction pattern of AgNPs derived from C. carandas fruit extract.*

°, 77.31 ° which corresponds to the planes (111), (200), (220) and (311) miller indices for AgNPs synthesized from fruit extract of *C. carandas*. The obtained results were matched with JCPDS file no. 04–0783, which indicates that the AgNPs is face-centered cubic (fcc) and crystalline in nature [21,22]. Further, the size of the AgNPs was calculated by Debye–Scherrer's equation using value at (111) plane (FWHM), which showed the average size of the AgNPs was found to be 45 nm. Similar results were reported by Ravichandran et al. [23] using an aqueous extract of *Callistemon lanceolatus*.

### 3.4. HR-TEM analysis

The size and shape of the AgNPs synthesized by fruit extract of *C. carandas* were carried out by HR-TEM analysis (Fig. 4). The HR-TEM image clearly showed that the synthesized AgNPs were spherical (with lattice fringes) in shape and size ranges between 10 and 50 nm (Fig. 4c and d). The image of HRTEM suggested that most of AgNPs were agglomerated and polydispersed (Fig. 4a and b). It was evident from the results that the size of AgNPs of HR-TEM was gently correlated with the size of the XRD analysis. A similar size (50 nm) and shape (spherical) of AgNPs have been reported by Anandalakshmi et al. [24].

### 3.5. Antioxidant assay of AgNPs

In the present investigation, the free radical scavenging activity of *C. carandas* fruit extract-mediated AgNPs was evaluated by DPPH method (Fig. 5). For antioxidant activity, different concentrations *viz,* 50, 100, 150, 200, and 250 μg/ml of the fruit extract of AgNPs against ascorbic acid (standard) and fruit extract were determined. Many studies have shown the highest antioxidant activity of AgNPs than the aqueous leaf extracts of medicinal plants [13,25]. From the results it was confirmed that the antioxidant activity of AgNPs has IC50 = 946 μg/mL while ascorbic acid has IC50 = 532 μg/mL and aqueous fruit extract

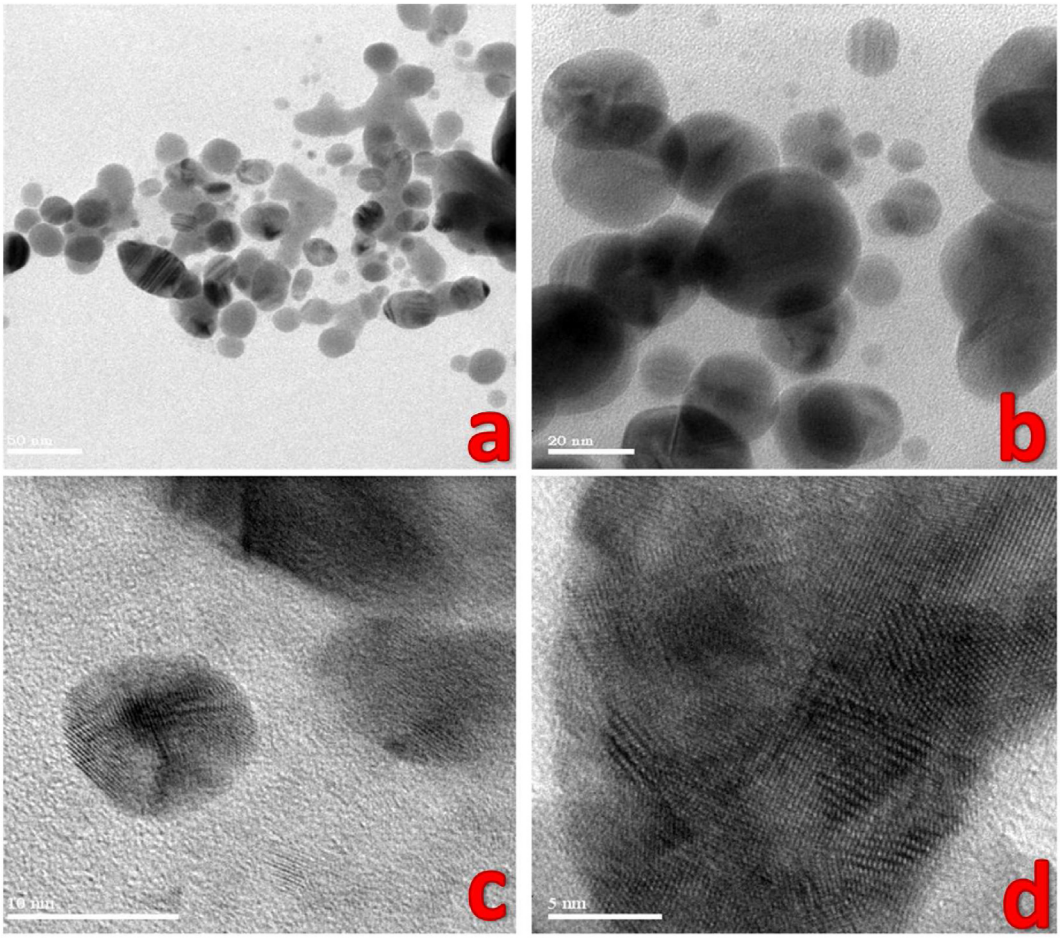


*Fig. 4. High resolution transmission electron microscope (HRTEM) of C. carandas fruit mediated AgNPs (a, b) TEM images, (c, d) HR-TEM images.*

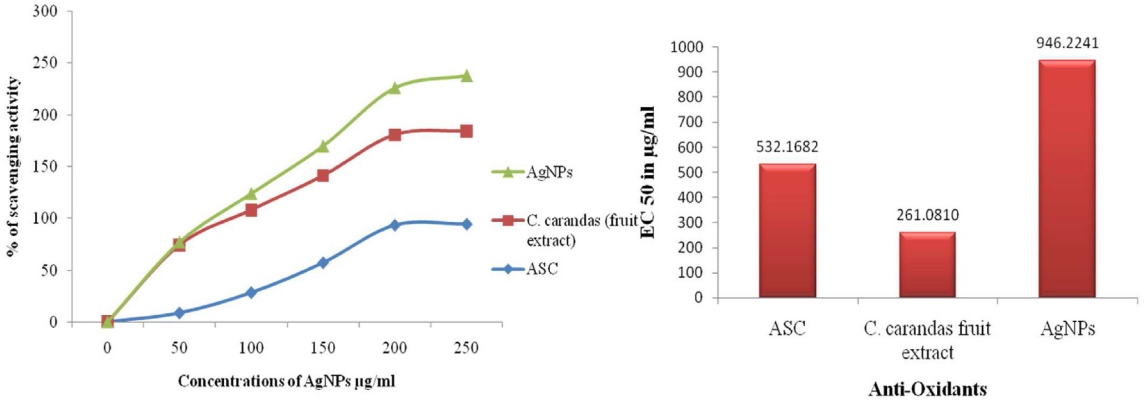


*Fig. 5. Antioxidant activity of AgNPs fabricated from fruit extract of C. carandas.*

(control) has IC50 = 261 µg/mL. The results suggest that the aqueous fruit extract itself is responsible for antioxidant activity and AgNPs enhances more antioxidant activity. The accumulation of flavonoids in the fruit extract is also responsible for highest antioxidant activity of AgNPs [26,27]. According to Afreen et al. [28], during the synthesis of flavonoids and other functional groups can cap on the surface of NPs resulting bioreduction of silver ions and stabilization of AgNPs.

### 3.6. Evaluation of antibacterial activity of AgNPs

The antibacterial activity of AgNPs fabricated from fruit extract of *C. carandas* was determined by agar well diffusion method (Fig. 6 and Table 2). The antibacterial activity was done with two different bacterial strains: *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Aqueous fruit extract containing

*Table 2. Antibacterial activity of AgNPs synthesized by fruit extract of C. carandas.*

| Concentrations of AgNPs (µg/ml) | Bacterial strains | |
|---|---|---|
| | Zone of inhibition (in mm) | |
| | *Escherichia coli* | *Staphylococcus aureus* |
| Fruit extract (control) | $0.000 \pm 0.000^{d}$ | $0.000 \pm 0.000^{e}$ |
| 25 | $0.000 \pm 0.000^{d}$ | $4.100 \pm 0.057^{d}$ |
| 50 | $2.966 \pm 0.033^{c}$ | $6.033 \pm 0.033^{c}$ |
| 100 | $7.100 \pm 0.057^{b}$ | $8.000 \pm 0.000^{b}$ |
| 250 | $10.000 \pm 0.028^{a}$ | $11.000 \pm 0.019^{a}$ |
| $AgNO_3$ | – | – |

The results are triplicates and were expressed in one way ANOVA as Mean ± SE with significant at $p < 0.05$.

AgNPs showed activity in all concentrations of silver tested against two bacterial strains. Whereas aqueous fruit extract (control) has not shown any activity. The zone of inhibition of AgNPs fabricated from *C. carandas* fruit extract against *S. aureus*

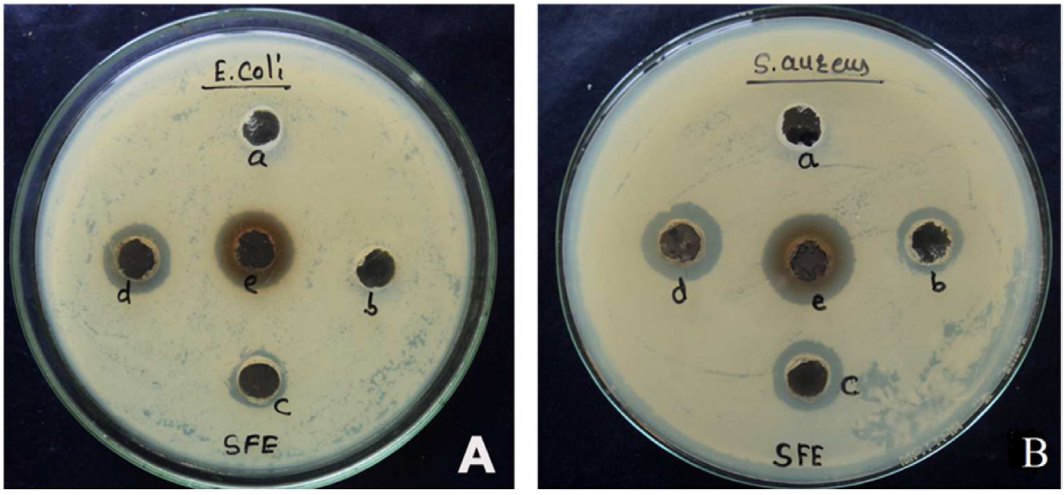


*Fig. 6. Antibacterial activity of AgNPs fabricated from fruit extract of C. carandas A) inhibition zone activity of E. coli B) inhibition zone activity of S. aureus.*

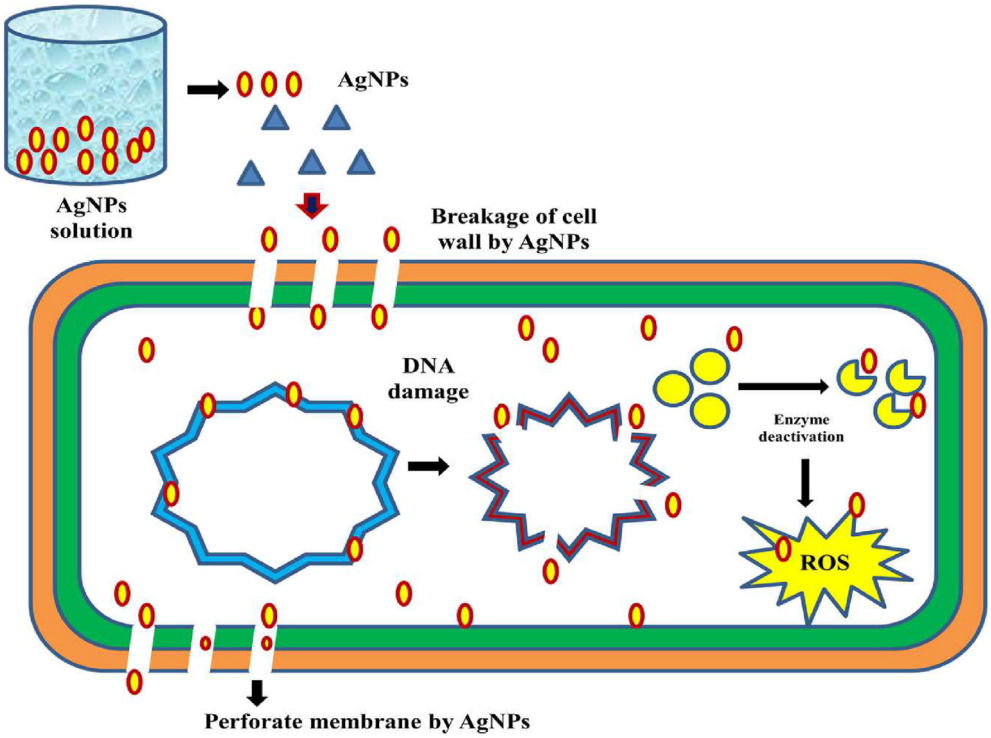


*Fig. 7. Schematic diagram showing toxicity mechanism of AgNPs against bacteria.*

($11.000 \pm 0.019^{a}$) exhibits the highest activity and the least activity was observed in *E. coli* ($10.000 \pm 0.028^{a}$) at 250 μg/ml respectively. It is evident from the results that only *E. coli* strain was susceptibility to the AgNPs in a dose-dependent manner. The fruit extract and $AgNO_3$ did not reveal any zone of inhibition activity. Moreover, gram-positive bacteria showed the highest antibacterial activity when compared to gram negative bacteria. The AgNPs derived from silk sericin are excellent growth inhibitors of firm bacteria [29]. Aritonang et al. [30] reported that the AgNPs fabricated from leaf extracts of *Lantana camara* and *Impatiens balsamina* showed good antibacterial activity against bacterial strains. The agglomeration of nanoparticles shows less zone of inhibition activity when compared to polydispersed AgNPs.

The mechanism of AgNPs against bacterial cells has not yet been completely understood. Silver salts inhibit the growth of bacteria in the human system. The bacterial cell wall when come in contact with the AgNPs. The AgNPs release silver ions continuously on bacterial cell envelope that leads to disruption of bacterial casings which might be considered as the mechanism of AgNPs [31] presented in Fig. 7. The uptake of silver ions causes different issues related to DNA including DNA replication, delayed cell cycle, cell propagation, and others. On the other hand, the cell wall bacteria can easily accumulate AgNPs based on their size [32]. The smaller nanoparticles (12 nm–30 nm) are involved in the pit formation on the cell wall of bacteria resulting trigger certain metabolic functions of bacteria which includes the production of ROS, deactivation of respiratory enzymes and interruption in ATP release [33]. The generation of ROS activity in a living system can damage cellular membrane; inhibit growth, and leads to the death of bacteria [34].

The AgNPs are likely to damage structural proteins (containing sulfur) and DNA (containing phosphorous) present in the membrane [35]. Additionally, AgNPs might inhibit protein synthesis by mortifying ribosomal components and induce the mechanism of signal transduction which significantly causes cell death or necrosis [33]. Researchers have studied AgNPs as antibacterial agents in several biomedical applications. This paper provides an overview mechanism of AgNPs and their potential antioxidant and antibacterial activity which could practice in future clinical treatment.

## 4. Conclusion

The present investigation reported on fabrication of AgNPs from fruit extract of *C. carandas* via eco-friendly method. The AgNPs synthesized fruit extract containing flavonoids are involved in the

bioreduction, biocapping and stabilization of AgNPs confirmed by FTIR analysis. The size (10 nm–95 nm) and spherical shaped AgNPs were determined by XRD and HR-TEM analysis. Further, AgNPs shows good antioxidant activity when compared with ascorbic acid (standard) and fruit extract (control). Finally, antibacterial activity of AgNPs was tested with two different bacterial strains. The gram positive bacteria (*S. aureus*) exhibits highest zone of inhibition than gram negative bacteria (*E. coli*) has been reported. The potential of biommetic AgNPs may be further used in various biological sources to combat bacterial diseases with significant pharmacological effects.

## Conflict of interest

There is no conflict of interest.

## Acknowledgements

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