Asian Journal of Nanoscience and Materials 3 (2020) 58-66

Asian Journal of Nanoscience and Materials

Journal homepage: www.ajnanomat.com

Original Research Article

Biosynthesis of silver nanoparticles using leaf and bark extract of indian plant *carissa carandas*, characterization and antimicrobial activity

Satish B. Manjare[a,*], Sandip G. Sharma[a], Vijay L. Gurav[a], Mamata R. Kunde[a], Sneha S. Patil[a], Shankar R. Thopate[b,*]

[a] *Department of Chemistry, Ratnagiri Sub-Centre, University of Mumbai, P-61, MIDC-Mirjole, Ratnagiri 415639. M.S. India*

[b] *Department of Chemistry, Shree Sadguru Gangageer Maharaj Science Gautam Arts & Sanjivani Commerce College, Kopargaon, Dist-Ahmednagar 423601, M.S. India*

ARTICLE INFORMATION

Received: 27 July 2019
Received in revised: 23 August 2019
Accepted: 23 August 2019
Available online: 14 November 2019

DOI: 10.26655/AJNANOMAT.2020.1.6

KEYWORDS

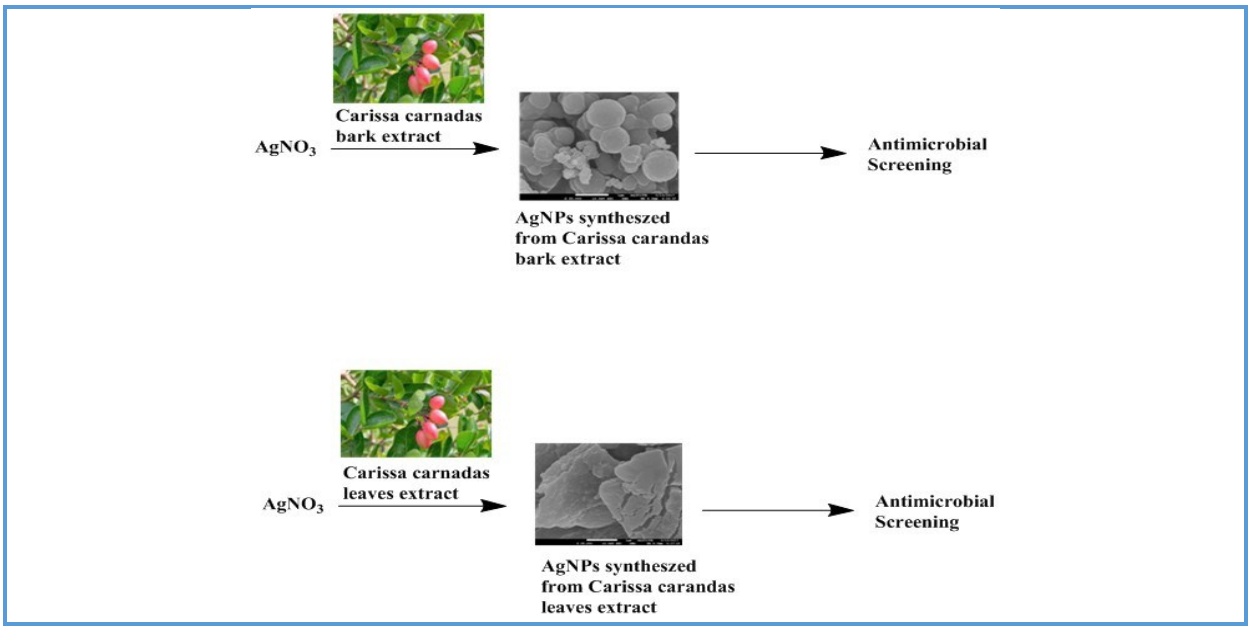
Green synthesis
Silver nanoparticles
UV-Visible Spectrophotometer
FT-IR
ICP-AES
Antimicrobial activity

ABSTRACT

Biosynthesized silver nanoparticle is a very expanding and useful area. The reductant material in the plant extracts (leaves and bark) of Carissa carandas can produce silver nanoparticles. The plant leaves and bark extract of Carissa caranadas act as reducing and capping agent. Conventionally, chemical reduction is the most frequently applied approach for preparation of metallic nanoparticles; however, it might be hazardous to environment. In the present work we report eco-friendly, cost effective, and green approach for the synthesis of AgNPs by using 0.02 M $AgNO_3$ solution and plant extracts (leaves and bark) of Carissa caranadas as reducing and capping agent. The synthesized nanoparticles were characterized using UV-VIS spectrophotomer, XRD, FT-IR, FE-SEM, and ICP-AES analysis. The biosynthesized silver nanoparticles showed a comparable antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli,* and *Aspergillus niger*. Antimicrobial activity of the biosynthesized silver nanoparticles suggests their possible application in medical and pharmaceuticals industry.

Corresponding author, email: satish.manjare@rediffmail.com (S.B. Manjare).
Tel.: +919881954889.

Graphical Abstract



Introduction

The application of nanoscale materials, particles, materials and structures, usually ranging from 1 nm to 100 nm, is a starving area of nanoscience and nanotechnology. Traditional chemical methods of synthesizing nanoparticles include the use of ethylene glycol [1], polyvinyl pyrrolidine (PVP) [2–5], and sodium borohydride [6–8]. The chemicals used in these methodologies can be toxic and highly reactive posing a risk to the environment and humans. The procedures are too expensive to be feasible at an industrial scale. Therefore, the economical and ecofriendly methods have become the need of the day. Incidentally, biological systems have been known to reduce metal ions into nano-sized particles with specific characteristic [9] and many researchers in chemistry, physics and engineering have recently used the biogenic synthesis of silver, gold, and palladium nanoparticles using a wide range of biological resources like plants [10, 11]. In the synthesis of nanoparticles by using plant extracts, the reduction is very rapid, single step, eco-friendly, and it does not require any specific conditions such as physical and chemical methods. Chemicals methods used for nanoparticles synthesis and stabilization are toxic and allergic, leading to non-ecofriendly byproducts. Nowadays, anticancer activities of nano-sized silver, gold, and palladium nanoparticles have been evaluated against the variety of human cancer cells [12–15]. Recent research includes the biosynthesis of AgNPs using leaf extracts of pepper leaf broth [16], Azadirachta indica [17] and flower extract of marigold flower [18]. Karonda (Carissa carandas) is of the family Apolcyanacea. It is native to India and also grown widely in other parts of world including, Nepal, Afghanistan, South Africa, Malaysia, Indonesia, Srilanka, and Australia. In India, it grows wildly in the states of Maharashtra, Uttar Pradesh, Bihar, Uttarakhand, Rajasthan, lower, outer and middle Himalayas and parts of southern India. The presence of natural antioxidants in *Carissa carandas* prompted us to use its leaf and bark extract as a suitable source for biosynthesis of nanomaterials. In this

research study, we report the biosynthesis of AgNPs using *Carissa carandas* leaves and bark extract. To the best of our knowledge, there is no report on the biosynthesis of the AgNPs using *Carissa carandas* leaves and bark extract as a stabilizing and reducing agent.

Experimental

Materials and Methods

All the chemicals were purchased from Merck, Ratnagiri, Maharashtra, and India, and used without purification. Dried leaves and bark of Carissa caranadas were collected from MIDC, Mirjole, Ratnagiri, MS, India. At the first, they were air-dried in sunlight.

Preparation of leaves and bark extract

Preparation of carissa caranadas *leaf extract*

10 g of the air-dried powdered of Carissa caranadas leaves was added to 100 mL of distilled water and then the mixture was boiled slowly for 20 min. The mixture was cooled to room temperature and filtered through filter paper then it was centrifuged for 20 min. The supernatant liquid was collected. This procedure was repeated twice. The filtrate was stored in refrigerator at 4 °C for further experiments. It was used as a reducing and stabilizing agent for 0.02 M of $AgNO_3$.

Preparation of carissa caranadas *bark extract*

10 g of the air-dried powdered of Carissa caranadas bark was added to 100 mL of distilled water and the mixture was boiled slowly for 20 min. Then the mixture was cooled and filtered through filter paper and then it was centrifuged for 20 min. The supernatant liquid was collected. This procedure was repeated twice. It was used as a reducing and stabilizing agent for 0.02 M of $AgNO_3$.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles from carissa caranadas *leaves extract*

The extract of the Carissa caranadas leaves (10.0 mL) was mixed with 27.5 mL of 0.02 M silver nitrate ($AgNO_3$) solution in 1:2.78 ratio, in a conical flask of 100 mL capacity under aseptic condition. Then the conical flask was kept inside the microwave oven at 400 °C for 6 min under the dark condition. It was then noticed that solution turned from yellowish to dark brown color indicating the formation of silver nanoparticles.

Synthesis of silver nanoparticles from carissacaranadas *bark extract*

The extract of the Carissa caranadas bark (10.0 mL) was mixed with 27.5 mL of 0.02 M silver nitrate ($AgNO_3$) solution in 1:2.78 ratio, in a conical flask of 100 mL capacity under aseptic condition. Then, the conical flask was kept inside the microwave oven at 400 °C for 6 min in dark condition. It was then noticed that the solution turned from yellowish to dark brown color, indicating the formation of the silver nanoparticles.

UV-visible spectroscopy

The reduction of the silver cation (Ag^+) ions by the test plant extracts such as Carissa caranadas in the solutions and the formation of silver nanoparticles were characterized using the UV-visible spectroscopy. The UV-VIS spectra of these samples were measured using a SYSTRONICS VISISCAN 167 spectrophotometer with a resolution of 1 nm at the range of 200-700 nm. The double distilled water was used to adjust the baseline.

Characterization of AgNPs by *FT-IR*

FT-IR analysis of the dried AgNPs was carried out using a Brucker FT-IR at the range of 4000-400 cm^{-1} transmittance mode, operating at a resolution of 4 cm^{-1}.

Characterization of AgNPs by FE-SEM

Magnification and agglomeration and size of nanoparticles of the synthesized silver nanoparticles were analyzed using a field emission scanning electron microscope (JSM-7600F, IIT Powai, Mumbai).

Characterization of AgNPs by XRD

The crystalline nature, structure, and size of the biosynthesized silver nanoparticles were analyzed using a Rigaku, Japan (Solapur University, Solapur).

Characterization of AgNPs by ICP-AES

Element analysis was carried out using the inductively coupled plasma atomic emission spectroscopy (ICP-AES, SPECTRO Analytical Instruments GmbH, Germany, IIT, Powai, Mumbai).

Antimicrobial activity AgNPs by agar well diffusion method

The synthesized silver nanoparticles (from Carissa caranadas bark and leaves) were analyzed for their antimicrobial activity against the gram-negative *E. coli* and gram-positive *S. Aureus* bacteria and fungus *Aspergillus niger*. The antimicrobial activity of the synthesized silver nanoparticles was evaluated using the Agar well diffusion method. Nutrient agar medium was prepared by dissolving 5.7 g of the commercially available M. H. Agar medium (Hi Media) in 150 mL deionised water. The dissolved medium was autoclaved for sterilization under the15 lbs pressure, and at 121 °C for 15 min. Saline was prepared by mixing the 0.85 gm of NaCl in 100 mL distilled water. The mixture was added to Nutrient Agar (N.A.) [Hi Media] medium. The test organisms were grown on the nutrient agar slants and stored at 4 °C. The cultures were sub cultured periodically. The autoclaved molten M. H. Agar medium was allowed to cool down to 40-45 °C. Then the old culture of test bacteria was added to it, mixed well, and was poured in a sterile petri dish. The plates were kept for media solidification. Wells were cut using cork borer in petri dishes containing Mullar Hinton Agar medium which were seeded with 24 h culture of fungal strains. The 0.1 mL plant extracts (aqueous and ethanol) were added to the wells. The sterilized plates were kept for diffusion in the refrigerator for 30 min. The sterilized plates were then incubated at 37 °C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The sterile distilled water and 0.02 M silver nitrate ($AgNO_3$) were used as a control and Penicillin was used as the standard antibiotic.

Results and Discussion

Synthesis of silver nanoparticles by using carissa caranadas *bark extract*

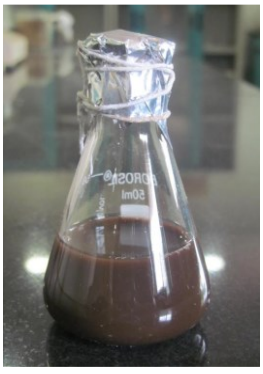
a) 0.02 M $AgNO_3$ solution before addition of bark extract

b) 0.02 M $AgNO_3$ solution & bark extract

Figure 1. Color change indicating synthesis of Nanoparticles Photographs of a) 0.02 M $AgNO_3$ without plant bark extracts, b) 0.02 M $AgNO_3$ with Carissa caranadas bark extract after 5 min

Synthesis of silver nanoparticles by using carissa caranadas *leaf extract*

a) 0.02 M $AgNO_3$ solution before addition of leaf extract



b) 0.02 M $AgNO_3$ solution & leaf extract

Figure 2. Color change indicating synthesis of Nanoparticles Photographs of a) 0.02M $AgNO_3$ without plant extracts, b) 0.02M $AgNO_3$ with Carissa caranadas leaves extract after 5 min

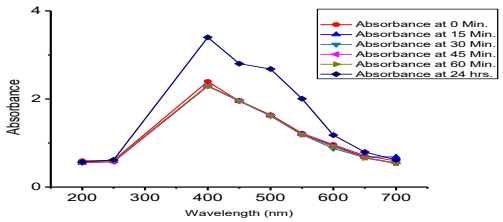


Figure 3. UV-Visible spectra of Silver Nanoparticles by using Carissa caranadas leaf extract

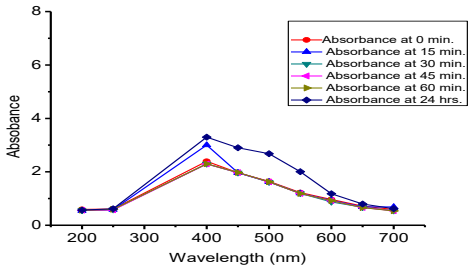


Figure 4. UV-Visible spectra of Silver Nanoparticles by using Carissa caranadas bark extract.

UV-vis analysis

The addition of Carissa caranadas leaf and bark extract to the silver nitrate ($AgNO_3$) solution, changed the color of the solution from transparent clear to colloidal brown, which was due to the production of silver nanoparticles. Figure 3 and 4 reveal the UV-VIS spectra from the reaction medium after 5 h. Absorption spectra of SNPs formed in the reaction media depicted the absorbance peak at 400-450 nm for the Carissa caranadas extracts. The broadening of peak in UV indicated that the particles are polydispersed and it shows surface plasmon resonance (SPR) phenomenon.

FT-IR analysis

The FT-IR spectrum of silver nanoparticles obtained using Carissa caranadas bark extract and leaves extract is shown in Figure 5 and 6.

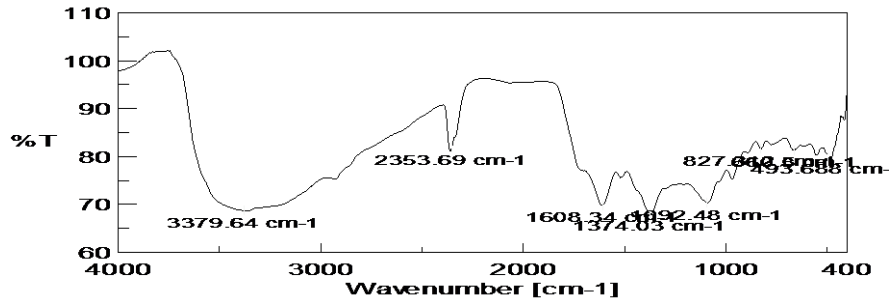


Figure 5. FTIR result for 1:100 ratio of silver nanoparticles of Carissa caranadas bark extract

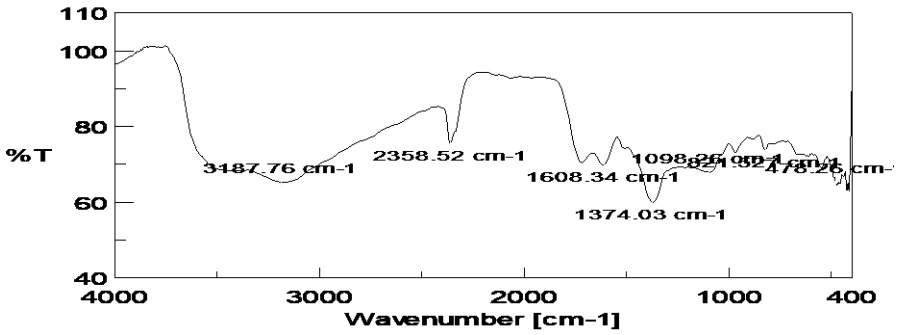


Figure 6. FT-IR result for 1:100 ratio of silver nanoparticles of Carissa caranadas leaves extract

The FT-IR spectrum (Figure 5) of silver nanoparticles of Carissa caranadas show strong IR bands characteristic peaks around 1092.48, 1374.03, 1608.34, 2358.69 cm^{-1}, and 3379.64 cm^{-1}. The observed peaks denote the presence of -C-O-C-, ether linkages, -C-N of aromatic amines, -C=C- group aromatic –C=C- stretch, -NH_2 and -OH groups stretch, respectively. The FTIR spectrum of the silver nanoparticles of Carissa caranadas in Figure 6 show strong IR bands characteristic peaks around 1098.26, 1374.03, 1608.34, 2358.52, and 3187.76 cm^{-1}. The observed peaks denote the presence of -C-O-C-, ether linkages, -C-N of aromatic amines, -C=C- group aromatic –C=C- stretch, -NH_2 and -OH groups stretch, respectively. These bands denote stretching and vibrational bands of compounds like alkaloids, terpenoids, and polyphenols present in the extract may be responsible for efficient capping and stabilization of obtained AgNPs [9].

FE-SEM analysis

The FE-SEM images in Figure 7 and 8 show very nice spherical particle with particle size ranging from 45 nm to 80 nm. The details regarding applied voltage, magnification used and the size of the contents of the images are implanted on the images themselves.

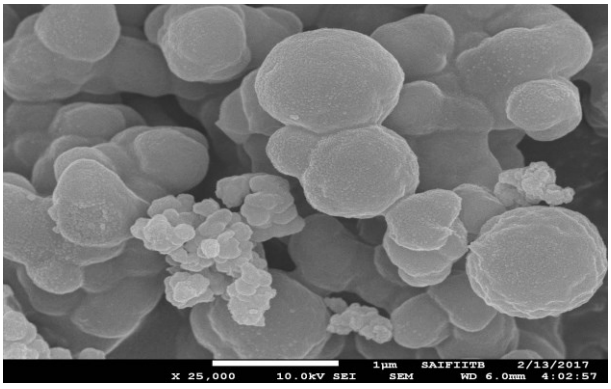


Figure 7. FE-SEM of silver nanoparticles formed by reaction of 0.02 M $AgNO_3$ and bark extract of Carissacaranadas

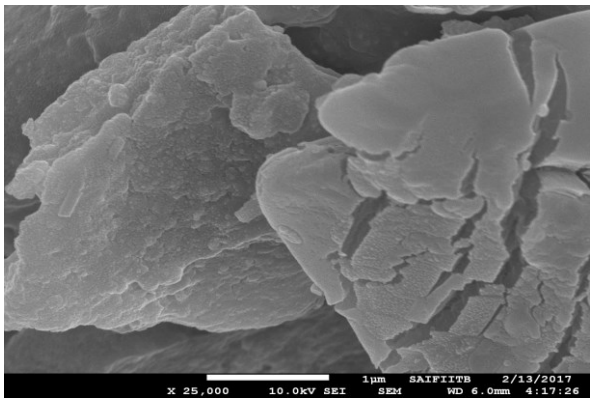


Figure 8. FE-SEM of silver nanoparticles formed by reaction of 0.02 M $AgNO_3$ and leaves extract of Carissa caranadas

XRD analysis

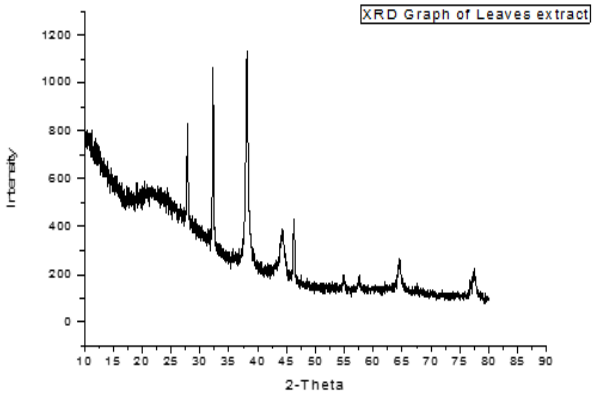


Figure 9. XRD analysis of silver Nanoparticles by using Carissa caranadas leaves extract

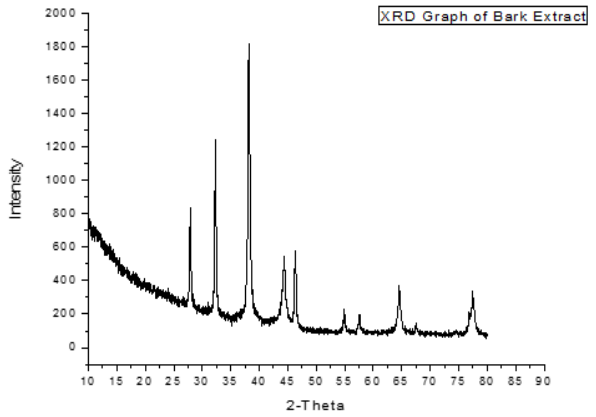


Figure 10. XRD analysis of Silver Nanoparticles by using Carissa caranadas bark extract

The XRD patterns obtained for the dried nanoparticles synthesized at different temperatures are shown in Figure 9 and Figure 10. To determine the crystalline nature, the size of the nanoparticles and nature of the compounds involved in the stabilization of nanoparticles. The obtained XRD patterns showed the crystalline and spherical nature of the synthesized silver nanoparticles. Four diffraction peaks were observed at 38.32, 44.90, 65.00 and 78.39^{0}showed at (111), (200), (220) and (311), reflections and the face-centered cubic structure of metallic silver, respectively (JCPDS No. 04-0783). The size of silver nanoparticles was calculated using the Scherrer equation.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where D is the crystallinity size (A^0), λ is X-ray wavelength in (A^0), β is the full width at half maximum (red), θ is Bragg diffraction angle, and k is constant (0.94). The size of nanoparticle was found to be at the range of 45-80 nm. The crystallite size values obtained from XRD are found to be within the range of the particle size obtained by FE-SEM analysis.

ICP-AES analysis

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis showed 15.85% Ag is present in the silver nanoparticles synthesized by Carissa caranadas leaves extract. And similarly inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis showed 23.41% Ag is present in the silver nanoparticles synthesized by Carissa caranadas bark extract.

Antimicrobial property analysis

In this study, the antimicrobial activity of AgNPs was observed by growing E. coli, S.

aureus and A. niger colonies on nutrient agar plates suspended with AgNPs. A control plate was separately maintained for above organisms in water, and the results are demonstrated in Table 1. The inhibition zones obtained exhibited considerable antibacterial activity and antifungal activity of the prepared silver nanoparticles. Results obtained in the previous research papers [17, 18] also support the antibacterial and antifungal potential of AgNPs. In the present study, Gram positive and Gram negative bacterial and fungal cultures were chosen for the antimicrobial activity of two selected parts of plant extracts including, leaves and bark. The results showed that the AgNPs of Carissa caranadas parts of plant extracts have significant antimicrobial activities against tested organism. Study of the antimicrobial activity indicated that antimicrobial activity of silver nanoparticles prepared from Carissa caranadas extracts exhibited a lower zone of inhibition when compared to standard antibiotic penicillin alone (Table 1).

Table 1. Antimicrobial Activity (Antibacterial and antifungal)

Test Organism	Zone of Inhibition (mm)			
	Leaves AgNPs	Bark AgNPs	Standard	Control
E. coli	0.2	0.4	1.4	+++
S. aureus	0.4	0.6	4.1	+++
A. niger	0.4	0.5	2.3	+++

Conclusion

In this work, synthesis of silver nanoparticles (AgNPs) by using Carissa caranadas is rapid, single step, eco-friendly, and is a significant alternative to the chemical methods. The synthesized AgNPs were characterized using the UV-VIS spectroscopy, FTIR, XRD, ICP-AES, and FE-SEM analysis. The FTIR analysis revealed the presence of organic molecules in the extracts which are responsible for the efficient reducing, capping and stabilization properties of these AgNPs. The synthesized AgNPs showed higher inhibitory effect against the *Escherichia coli, Staphylococcus aureus,* and Aspergillus niger compared with that of the crude leaf and bark extract. The synthesized AgNPs depicted the antimicrobial activity with good results which could be of immense use in the medicinal field for their efficient antimicrobial function.

Acknowledgements

The authors should like to thank BCUD Mumbai University for financial assistance (Proposal Number-277).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1]. Li X., Zhu T., Shao Z., Li Y., Chang H., Gao W., et al. *Tetrahedron*. 2016, **72**:69
[2]. Gurunathan S. *Arab J Chem*., 2019, **12**:168
[3]. Banerjee P., Satapathy M., Mukhopahayay A., Das P. *Bioresour Bioprocess*. 2014, **1**:1
[4]. Nemamcha A., Moumeni H., Rehspringer J.L. Phys. Procedia., 2009, **2**:713
[5]. Sharma M., Sarma P.J., Goswami M.J., Bania K.K. *J Colloid Interface Sci*., 2017, **490**:529
[6]. De Castro K.A., Rhee H. *J Incl Phenom Macrocycl Chem*. 2015, **82**:13
[7]. Kora A.J., Rastogi L. *Arab J Chem*. 2018, **11**:1097

[8]. Santoshi kumari A., Venkatesham M., Ayodhya D., Veerabhadram G. *Appl Nanosci*. 2015, 5:315
[9]. Singhal G., Bhavesh R., Kasariya K., Sharma A.R., Singh R.P. *J Nanoparticle Res*., 2011, **13**:2981
[10]. Gavhane A., Padmanabhan P., Kamble S., Jangle S. *Int J Pharma Bio Sci*., 2012, **3**:88
[11]. Kalpana D., Han J.H., Park W.S., Lee S.M., Wahab R., Lee Y.S. *Arab J Chem* . 2014, **12**:1722
[12]. Krishnaraj C., Muthukumaran P., Ramachandran R., Balakumaran M.D., Kalaichelvan P.T. *Biotechnol Reports*., 2014, **4**:42
[13]. Deshmukh S.R., Ashrit D.S., Patil B.A. *Int J Pharm Pharm Sci*., 2012, **4**:329
[14]. Siddiqi K.S., Husen A. Nanoscale Res. Lett., 2016, **11**:482
[15]. Gurunathan S., Kim E.S., Han J.W., Park J.H., Kim J.H., Grumezescu A.M. *Molecules*. 2015, **20**:22476
[16]. Mallikarjuna K., John Sushma N., Narasimha G., Manoj L., Deva Prasad Raju B. *Arab J Chem*., 2014, **7**:1099
[17]. Satapathy M.K., Banerjee P., Das P. *Appl Nanosci*., 2015, **5**:1
[18]. Padalia H., Moteriya P., Chanda S. *Arab J Chem*., 2015, **8**:732

How to cite this manuscript: Satish B. Manjare*, Sandip G. Sharma, Vijay L. Gurav, Mamata R. Kunde, Sneha S. Patil, Shankar R. Thopate*. Biosynthesis of silver nanoparticles using leaf and bark extract of indian plant *carissa carandas*, characterization and antimicrobial activity. *Asian Journal of Nanoscience and Materials*, 3(1) 2020, 58-66. DOI: 10.26655/AJNANOMAT.2020.1.6