JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/36458

RESEARCH ARTICLE

Biofabrication of Silver Nanoparticles Using Green Seaweed: Characterisation and Antibacterial Evaluation

Nanthakumar Ramalingam[1*], Prabakaran Venkatachalam[2], Chellan Rose[3] and Seethalakshmi Sankar[4]

[1]Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai – 600116, Tamil Nadu, India; pharma_chemistry1980@rediffmail.com
[2]Novitium Pharma LLC, East Windsor, New Jersey – 08520, USA
[3]Department of Biotechnology, CSIR – Central Leather Research Institute, Adyar, Chennai – 600020, Tamil Nadu, India
[4]Department of Pharmacology, ESI Medical College and PGIMSR, K. K. Nagar, Chennai – 600012, Tamil Nadu, India

Abstract

This study investigates the *Chaetomorpha antennina* (CA) seaweed-mediated biosynthesis of silver nanoparticles (AgNPs). The synthesis process of silver nanoparticles was monitored over time with the help of an Ultraviolet-visible spectrophotometer and further characterisation studies were also performed. Differential Light Scattering (DLS) measurements revealed a mean particle size of approximately 103.5nm and a mean zeta potential value of -57.5mV for AgNPs. The spherical shape and size of the AgNPs were confirmed through High-Resolution Transmission Electron Microscopy (HR-TEM) imaging, while Energy Dispersive X-ray Spectroscopy (EDAX) analysis provided insights into the elemental composition. The concentration of AgNPs was estimated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The antibacterial potential of AgNPs was evaluated against both gram-positive (*Bacillus cereus*, *Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacterial strains (*Klebsiella pneumoniae, Escherichia coli*, *Shigella dysentriae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) using the agar well diffusion method. From the results, AgNPs exhibited significant antibacterial activity against *B. subtilis* and *S. typhi* among all the tested concentration levels (25, 50, 75 and 100µl).

Keywords: Antibacterial Activity, Characterisation, Green Seaweed, Silver Nanoparticles

1. Introduction

Synthesising Metal Nanoparticles (MNPs) stands out as an emerging frontier in nanotechnology research. Within this domain, researchers leverage a spectrum of methods encompassing physical, chemical and biological approaches to produce MNPs. Biological methods are advantageous over the other methods due to less reaction time, cost-effectiveness, eco-friendliness, the stability of nanoparticles etc. In the biological method, various resources such as bacteria, fungi, terrestrial plants, marine sources[1], honey[2], gripe water[3], isolated phytoconstituents[4] and Ayurvedic formulations[5] are used in the synthesis. Many of the plants and different parts of the plants[6-8] are exploited in metal nanoparticle synthesis. Moreover, the AgNPs synthesised from plant sources are also made into different formulations[9,10]. The ocean possesses enormous sources of bioactive compounds. Recently, several marine sources such as seaweeds[11,12], seagrasses, mangroves[13], sponges[14], bivalves[15] etc., were also explored to synthesise AgNPs. Our review provides a comprehensive analysis of the generation of silver and gold nanoparticles using marine sources, along with the various techniques employed for their characterisation[16]. Interestingly, except for a few reports, marine algae are not thoroughly investigated

*Author for correspondence

Article Received on: 04.02.2024 Revised on: 29.02.2024 Accepted on: 01.03.2024

in MNP synthesis. This aroused our interest in the present research work.

Chaetomorpha antennina is a green marine alga belonging to the family of Cladophoraceae. Polyherbal preparations were made using *C. antennina* with other marine plants and evaluated for antiplasmodial activity[17]. Petroleum ether extract of *C. antennina* was prepared and screened for antibacterial activity against various bacterial strains[18]. The current research employed an aqueous extract from green algae to fabricate AgNPs. The AgNPs formed were characterised, their concentration was determined with ICP-OES and their antibacterial potential was determined.

2. Materials and Methods

2.1 Biofabrication of Silver Nanoparticles (AgNPs)

The plant was obtained in the coastal area of Rameswaram, Tamilnadu, India. The newly collected seaweed was rinsed with seawater, followed by millipore water to eliminate other extraneous matter. It was left to dry in the shade for a duration of 10 to 15 days and then coarse powder of the seaweed was prepared using a mixer. An aqueous extract of the seaweed was obtained by boiling the coarse powder with millipore water. After centrifugation at 10,000 rpm, the extract underwent filtration with Whatman filter paper No. 1 to achieve a clear solution. 1mM silver nitrate was prepared using millipore water and mixed with the CA extract. The production of nanoparticles was visually monitored in the initial stages.

2.2 Characterization of AgNPs

UV-visible spectrophotometry (Shimadzu UV-2450, Japan) was employed to monitor AgNP formation at regular intervals, covering the spectral range of 300-800nm. The zeta potential and the size of the nanoparticles were determined using a particle size analyser (Horiba Scientific, SZ-100). HR-TEM (JEOL 3010) recorded the particle size, shape and internal surface morphology of the AgNPs. EDAX (Oxford Instruments) spectrum was also recorded to confirm the presence of silver. ICP-OES (Perkin Elmer Optima 5300 DV) was employed to estimate the amount of AgNPs present.

2.3 Antibacterial Activity

The study evaluated the antibacterial properties of CA-mediated AgNPs using the agar well diffusion method[19,20] against a range of gram-positive bacteria like *B. cereus*, *S. aureus* and *B. subtilis* and gram-negative bacteria like *K. pneumoniae*, *E. coli*, *S. dysentriae*, *S. typhi*, *P. aeruginosa* and *P. mirabilis* bacterial strains. Nutrient agar medium was used for culturing bacterial cultures. The bacterial culture was uniformly distributed onto the solidified agar plates using a glass spreader after sterile nutrient agar plates were made. All the procedures were carried out in a laminar airflow chamber. The bacterial cells had enough time to stick together. Four wells of appropriate diameter were created in the solidified agar with a cork borer. Utilising a sterile micropipette, varying volumes of AgNPs such as 25, 50, 75, and 100 µl were dispensed into the wells. The plates were then placed in an incubator at 37°C for 24 hours. Later, each concentration's zone of inhibition was measured and reported.

3. Results and Discussion

This study explored CA-mediated simple, cost-effective, eco-conscious synthesis of silver nanoparticles. At room temperature, the marine algae extract prepared with water was combined with a silver nitrate solution (Figure 1a). The Biogenesis of AgNPs was initially monitored by visual inspection. The colourless solution turned to a pale reddish-brown colour (Figure 1b), indicating the initiation of AgNPs formation. As time progressed, the intensity of the colour also increased. The ultraviolet-visible spectra of the sample (Figure 2a) were

Figure 1. Green synthesis of AgNPs using *Chaetomorpha antennina* extract: (a) Control (b) Silver nanoparticles synthesized using CA.

captured, revealing a λ_{max} at 424.5nm, a characteristic feature of AgNPs[21]. Additionally, ultraviolet-visible spectra were recorded at various time intervals and the overlaid spectra were depicted for comparison (Figure 2b). DLS studies were conducted to analyse the average particle size and stability of the synthesised AgNPs. The average particle size of silver nanoparticles (Figure 3) produced through CA was determined to be 103.5nm. Furthermore, the zeta potential of AgNPs (Figure 4) was measured at -57.5mV suggesting a high degree of particle stability[22]. The existence of silver and other elements was confirmed by the EDAX profile. EDAX (Figure 5) results showed the presence of silver with other elements, particularly carbon and oxygen. Additional minor peaks were detected, possibly attributed to organic material from the seaweed[23]. The morphology and size of the AgNPs were analysed using HR-TEM and the results showed that the particles were spherical (Figures 6a, 6b, and 6c). In HR-TEM images, a layer was found over the AgNPs, which may be due to the presence of biomolecules from the seaweed, which is in agreement with EDAX results. Using ICP-OES, the concentration of AgNPs was determined to be 54.03mg/l at a wavelength of 328.068nm.

The antibacterial activity was tested using six gram-negative and three gram-positive strains of bacteria. Figure 7 illustrates the antibacterial capacity of AgNPs produced with CA across various concentration levels. The zone of inhibition of AgNPs against various bacterial strains at four different concentration levels is shown in Table 1. The findings of the study demonstrated that AgNPs were effective against all the examined gram-positive bacterial strains. In the case of gram-negative strains, AgNPs were effective only against *E. coli*, *S. typhi* and *P. aeruginosa*. It was ineffective against *K. pneumoniae*, *S. dysentriae* and *P. mirabilis*. AgNPs were effective against *B. subtilis* and *S. typhi* in all the dose levels tested (Figure 8). Dose-dependent responses were seen in both cases. AgNPs only demonstrated a zone of inhibition at 75µl and 100µl against *B. cereus*,

Table 1. Antibacterial activity of AgNPs synthesised using *Chaetomorpha antennina*

Test Organisms	Zone of Inhibition in mm at Different Concentrations			
	25 µl	50 µl	75 µl	100 µl
B. subtilis	14	15	19	25
B. cereus	-	-	12	15
S. aureus	-	-	12	15
E. coli	-	-	-	13
K. pneumoniae	-	-	-	-
S. typhi	15	17	20	23
S. dysentriae	-	-	-	-
P. mirabilis	-	-	-	-
P. aeruginosa	-	-	10	13

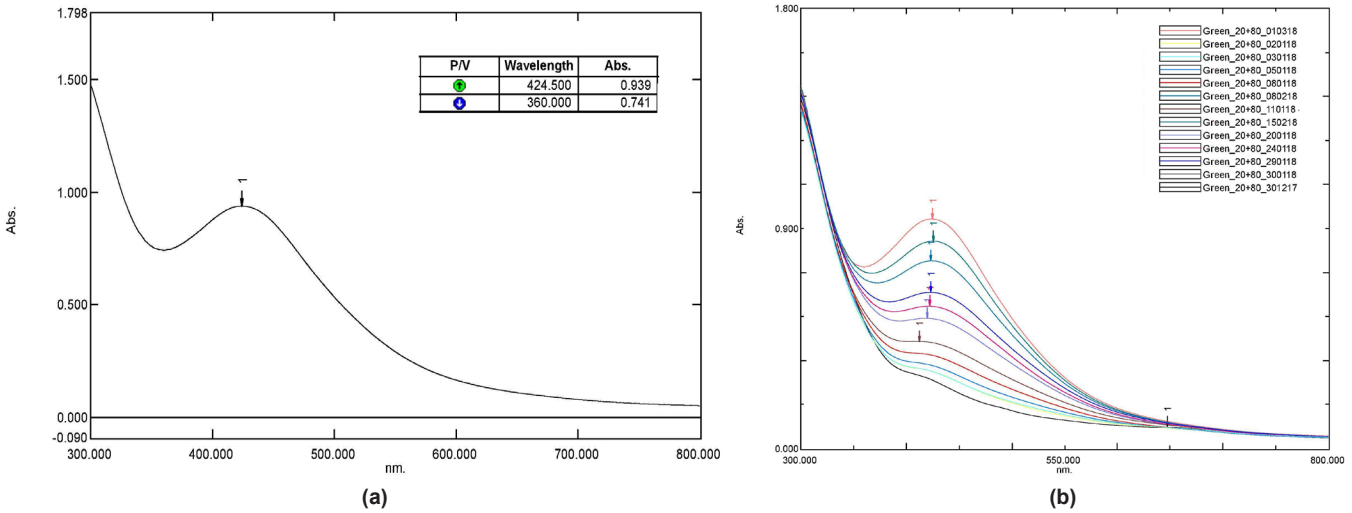

Figure 2. (a). Ultraviolet-visible spectra of AgNPs synthesized using *Chaetomorpha antennina*, (b). Ultraviolet-visible spectra of AgNPs synthesized using *Chaetomorpha antennina* at different time intervals.

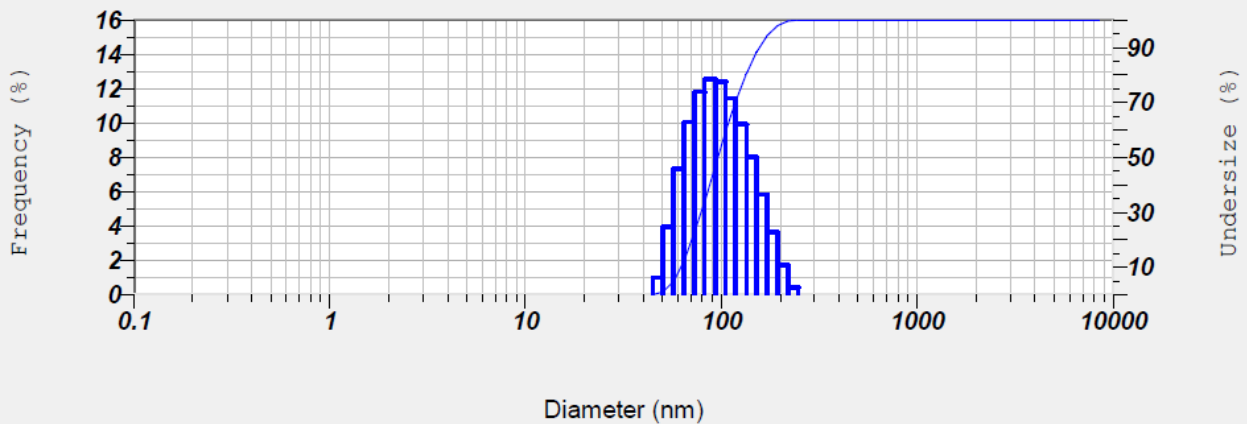

Figure 3. Distribution of particle size in AgNPs synthesized using CA.

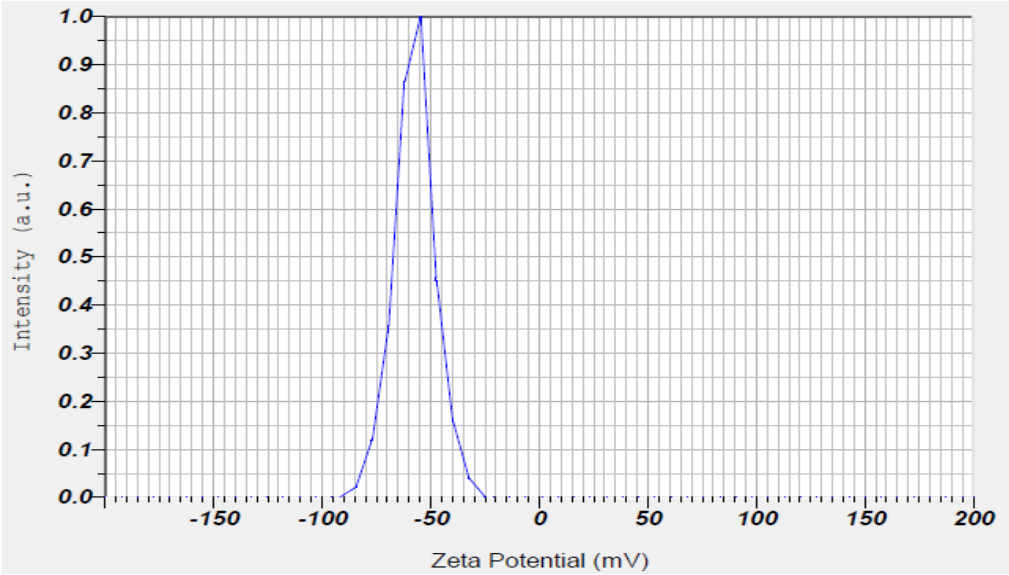

Figure 4. The measurement of Zeta potential for AgNPs synthesized using CA.

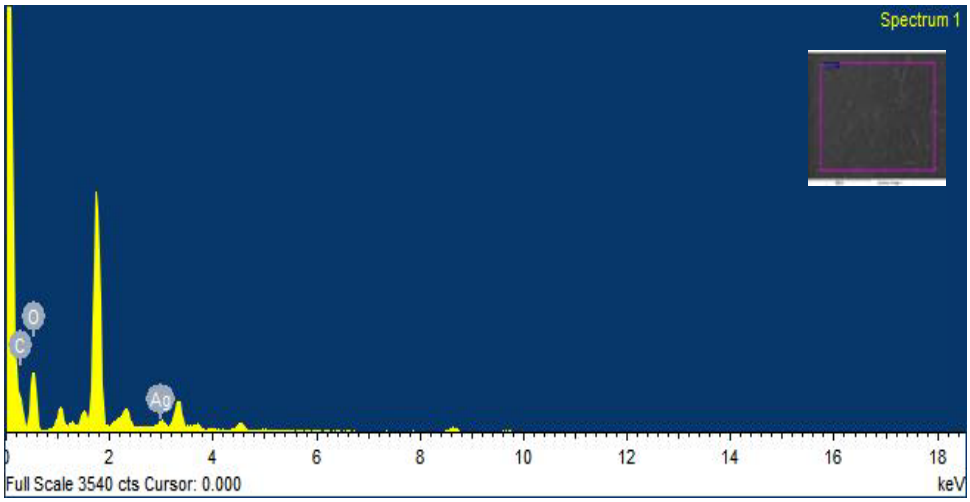

Figure 5. EDAX profile of CA-mediated AgNPs.

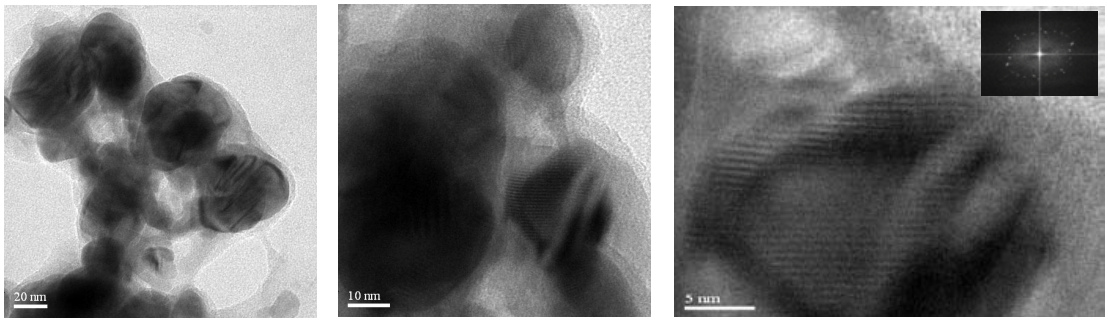

Figure 6. (a), (b), (c). HR-TEM images of CA-mediated AgNPs at different scales (inset showing FFT view).

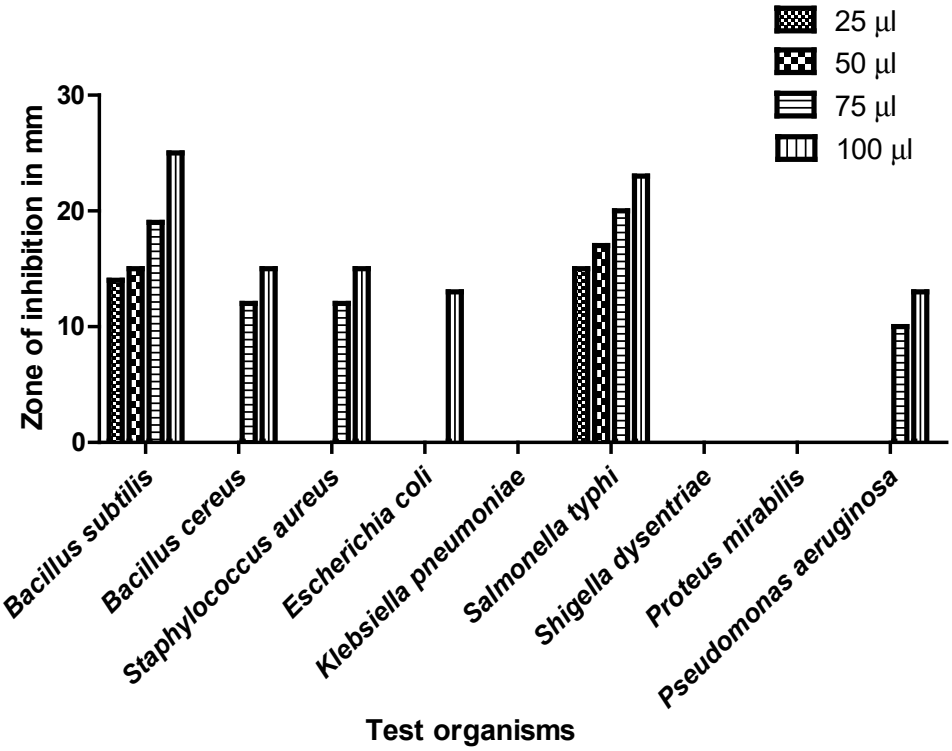

Figure 7. Antibacterial activity of *Chaetomorpha antennina*-mediated AgNPs at different concentrations.

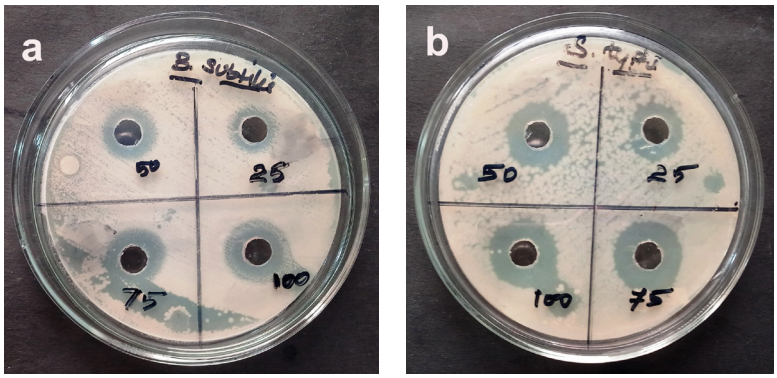

Figure 8. Antibacterial activity of *Chaetomorpha antennina*-mediated AgNPs against (a). *B. subtilis*, (b). *S. typhi* at 25, 50, 75 and 100 µl.

S. aureus and *P. aeruginosa*. AgNPs were only shown to exhibit antibacterial action against *E. coli* at the highest tested dose level of 100µl.

4. Conclusion

In the present study, a simple, stable, efficient, nature-friendly biofabrication of silver nanoparticles using *C. antennina* was explored. Various spectroscopic and microscopic analyses were employed to characterise the synthesised nanoparticles. These characterisation techniques offer complementary information about the physical, chemical and structural properties of synthesised nanoparticles, enabling a comprehensive understanding of their characteristics. The AgNPs were evaluated for their antibacterial efficacy, demonstrating their significant potency against gram-positive bacterial strains and effectiveness against selected gram-negative bacterial strains. Increasing the concentration of the AgNPs may elucidate their antibacterial potential against a broader spectrum of gram-negative bacterial strains. Nevertheless, CA-synthesized AgNPs may be used alone or in conjugation with other antibiotics for diseases caused by gram-positive organisms. The exact biomolecule involved in the bioreduction process can be identified in future studies and this can open many avenues in the biomedical applications of silver nanoparticles.

5. Acknowledgements

The authors thank the Management of Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, for providing the necessary facilities to conduct this research. The authenticity of *C. antennina* was confirmed by Mr J. R. Ramalingam, Retired technical officer from the Mandapam Regional Centre of Central Marine Fisheries Research Institute, Indian Council of Agricultural Research. Additionally, they would like to express their appreciation to the Centre for Nanoscience and Nanotechnology, a collaborative initiative of IGCAR, Kalpakkam, and Sathyabama Institute of Science and Technology (Deemed to be University), for their assistance in EDAX measurements. The authors also acknowledge the DST unit of the Nanoscience lab, Department of Chemistry, IIT Madras, for their contributions to HR-TEM measurements, as well as SAIF, IIT Madras, for their assistance with ICP-OES measurements. Furthermore, the authors extend their thanks to Prof. Basavaraj M. G. and Mr. P. Logesh Kumar of the PECS lab, Department of Chemical Engineering, IIT Madras, for their support in DLS analysis.

6. References

1. Abdelghany TM, Al-Rajhi AM, Al Abboud MA, Alawlaqi MM, Ganash Magdah A, Helmy EA, Mabrouk AS. Recent advances in green synthesis of silver nanoparticles and their applications: About future directions. A review. Bio Nano Sci. 2018; 8:5-16. https://doi.org/10.1007/s12668-017-0413-3
2. Philip D. Honey mediated green synthesis of silver nanoparticles. Spectrochim Acta Part A. 2010; 75(3):1078-81. https://doi.org/10.1016/j.saa.2009.12.058 PMid:20060777.
3. Kirubha E, Vishista K, Palanisamy PK. Gripe water-mediated green synthesis of silver nanoparticles and their applications in nonlinear optics and surface-enhanced Raman spectroscopy. Appl Nanosci. 2015; 5:777-86. https://doi.org/10.1007/s13204-014-0376-4
4. Hoshyar R, Khayati GR, Poorgholami M, Kaykhaii M. A novel green one-step synthesis of gold nanoparticles using crocin and their anti-cancer activities. J Photochem Photobiol B. 2016; 159:237-42. https://doi.org/10.1016/j.jphotobiol.2016.03.056 PMid:27085640.
5. Aromal SA, Babu KD, Philip D. Characterisation and catalytic activity of gold nanoparticles synthesised using ayurvedic arishtams. Spectrochim Acta Part A. 2012; 96:1025-30. https://doi.org/10.1016/j.saa.2012.08.010 PMid:22954810.
6. Ezealisiji KM, Noundou XS, Ukwueze SE. Green synthesis and characterisation of monodispersed silver nanoparticles using root bark aqueous extract of *Annona muricata* Linn and their antimicrobial activity. Appl Nanosci. 2017; 7:905-11. https://doi.org/10.1007/s13204-017-0632-5
7. Das M, Smita SS. Biosynthesis of silver nanoparticles using bark extracts of *Butea monosperma* (Lam.) Taub. and study of their antimicrobial activity. Appl Nanosci. 2018; 8:1059-67. https://doi.org/10.1007/s13204-018-0721-0
8. Aisida SO, Ugwu K, Akpa PA, Nwanya AC, Nwankwo U, Botha SS, Ejikeme PM, Ahmad I, Maaza M, Ezema FI. Biosynthesis of silver nanoparticles using bitter leave (*Veronica amygdalina*) for antibacterial activities. Surf Interfaces. 2019; 17:100359. https://doi.org/10.1016/j.surfin.2019.100359
9. Odeniyi MA, Okumah VC, Adebayo-Tayo BC, Odeniyi OA. Green synthesis and cream formulations of silver nanoparticles of *Nauclea latifolia* (African peach) fruit extracts and evaluation of antimicrobial and antioxidant

activities. Sustainable Chem Pharm. 2020; 15:100197. https://doi.org/10.1016/j.scp.2019.100197
10. Sood R, Chopra DS. Optimisation of reaction conditions to fabricate *Ocimum sanctum* synthesised silver nanoparticles and its application to nano-gel systems for burn wounds. Mater Sci Eng C. 2018; 92:575-89. https://doi.org/10.1016/j.msec.2018.06.070 PMid:30184784.
11. Ramalingam N, Rose C, Krishnan C, Sankar S. Green synthesis of silver nanoparticles using red marine algae and evaluation of its antibacterial activity. J Pharm Sci Res. 2018; 10(10):2435-8.
12. Kannan RR, Arumugam R, Ramya D, Manivannan K, Anantharaman P. Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. Appl Nanosci. 2013; 3:229-33. https://doi.org/10.1007/s13204-012-0125-5
13. Se-Kwom Kim. Springer handbook of marine biotechnology. Springer-Verlag Berlin Heidelberg. 2015; p. 1229-46.
14. Inbakandan D, Sivaleela G, Peter DM, Kiurbagaran R, Venkatesan R, Khan SA. Marine sponge extract assisted biosynthesis of silver nanoparticles. Mater Lett. 2012; 87:66-8. https://doi.org/10.1016/j.matlet.2012.07.083
15. Satapathy S, Paikaray S, Thirunavoukkarasu M, Panda CR, Subbudhi E. Biosynthesis and characterisation of silver nanoparticles derived from marine bivalve *Donax cuneatus* (Linnaeus) and assessment of its antimicrobial potential. Inorg Nano-Met Chem. 2017; 47(7):1044-8. https://doi.org/10.1080/24701556.2017.1284083
16. Ramalingam N, Rose C, Krishnan C, Sankar S, Kurian SG. Extracellular biofabrication of silver and gold nanoparticles: Treasures from the abyssal zone. Asian J Pharm Clin Res. 2019; 12(2):44-54. https://doi.org/10.22159/ajpcr.2019.v12i2.29781
17. Ravikumar S, Ramanathan G, Jacob Inbaneson S, Ramu A. Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. Parasitol Res. 2011; 108:107-13. https://doi.org/10.1007/s00436-010-2041-5 PMid:20844892.
18. Sivakumar SM, Safhi MM. Isolation and screening of bioactive principle from *Chaetomorpha antennina* against certain bacterial strains. Saudi Pharm J. 2013; 21(1):119-21. https://doi.org/10.1016/j.jsps.2012.02.003 PMid:23960826 PMCid: PMC3745066.
19. Pattanayak S, Mollick MM, Maity D, Chakraborty S, Dash SK, Chattopadhyay S, Roy S, Chattopadhyay D, Chakraborty M. *Butea monosperma* bark extract mediated green synthesis of silver nanoparticles: Characterisation and biomedical applications. J Saudi Chem Soc. 2017; 21(6):673-84. https://doi.org/10.1016/j.jscs.2015.11.004
20. Charulatha S, Dharrunya HV, Dhanamurugan R, Devadharshini R, Paul D, Sinduja ME, Bindhu J. Evaluation of antioxidant and antimicrobial potential of green synthesised Ag nanoparticles from ethanolic leaf extract of *Thespesia populnea*. J Nat Remed. 2022; 22(2):137-44. https://doi.org/10.18311/jnr/2022/26226
21. Elmusa F, Aygun A, Gulbagca F, Seyrankaya A, Göl F, Yenikaya C, Sen F. Investigation of the antibacterial properties of silver nanoparticles synthesised using *Abelmoschus esculentus* extract and their ceramic applications. Int J Environ Sci Technol. 2021; 18:849-60. https://doi.org/10.1007/s13762-020-02883-x
22. Dua TK, Giri S, Nandi G, Sahu R, Shaw TK, Paul P. Green synthesis of silver nanoparticles using *Eupatorium adenophorum* leaf extract: characterisations, antioxidant, antibacterial and photocatalytic activities. Chemical Papers. 2023; 77:2947-56. https://doi.org/10.1007/s11696-023-02676-9 PMid:36714039 PMCid: PMC9873543.
23. Mofolo MJ, Kadhila P, Chinsembu KC, Mashele S, Sekhoacha M. Green synthesis of silver nanoparticles from extracts of *Pechuel-loeschea leubnitziae*: Their anti-proliferative activity against the U87 cell line. Inorg Nano-Met Chem. 2020; 50(10):949-55. https://doi.org/10.1080/24701556.2020.1729191