INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 11, Issue 11- 2024

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2024.11.11.001

Biosynthesis of silver nanoparticles from *Hibiscus rosasinensis* and their antibacterial activities on *Vibrio* species isolated from pathogenic catfish

Asonye, C M*.,Ogbulie, J.N., Chinakwe, E. C and Braide, W
Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria
*Author for correspondence: **email:**chinemeremasonye2@gmail.com; +234-810 320 0435

Abstract

Bacterial pathogens isolated from fish seem to exhibit significant resistance to antibiotics, posing a challenge for both the economy and public health. Natural alternatives to conventional antibiotics are crucial in addressing antibiotic resistance. Nanotechnology has demonstrated significant promise across various research domains and the antimicrobial characteristics of silver nanoparticles are well documented. Silvers has been utilized for therapeutic purposes since ancient times due to its bactericidal characteristics and the high reactive surfaces of silver nanoparticles (AgNPs) review that they might be active (have a function) in antimicrobial applications. This work aimed to study the antibacterial activities of biologically produced AgNps from *Hibiscus rosa sinensis* (Hibiscus leaves) on *Vibrio* spp. Isolated from pathogenic catfishes. The nanoparticles were characterized by UV – Vis Spectrophotometry, Scanning Electron Microscopy and Fourier Transform Infra-red analysis and their antibacterial activities were tested against the *Vibrio* spp.isolated from diseased catfish samples by well-in-agar diffusion method using Gentamicin as the control antibiotic. Although Gentamicin was more effective than the nanoparticles against the *Vibrio* spp.,the results obtained declare that the biological synthesis of silver nanoparticles are safe and eco-friendly. Moreover, silver nanoparticles have interesting molar concentration and dose-dependent antibacterial properties; their effectiveness opens way to their use in place of conventional antibiotics to limit fish diseases, reduce antibiotic resistance, increase the economy and improve aquacultural and human health.

Keywords: Silver nanoparticles, Antibacterial activity, *Hibiscus rosa sinensis*, *Vibrio* spp., Pathogenic fish.

Introduction

In recent years, aquaculture has become increasingly crucial in meeting the high demand for animal protein and contributing to food security. Nevertheless, issues such as environmental pollution and the rise of diseases have become key challenges for aquaculture industry. Various types of bacteria found in fish can lead to illnesses, making them a major concern for the aquaculture industry. Outbreaks of these diseases can negatively impact both the quality and quantity of production, with fish mortality being a particular consequence. Consequently, due to this issue and rising disease incidence, there has been an increased dependency on antibiotics in aquaculture globally, especially in regions where regulatory guidelines are not well-defined or monitored. In the context of aquaculture, antibiotics are also employed to promote fish growth. The use of antibiotics for treating fish diseases is frequently not regulated in numerous developing countries, resulting in reckless usage of these drugs and the emergence of antibiotic resistance (Budiati *et al*., 2013).

Vibrio species are well-known pathogenic bacteria for fish (Abdel-Tawwab *et al*., 2010). They are highly motile, Gram-negative curved rod-shaped facultative anaerobes that include several species that are highly pathogenic to humans. Three species of *Vibrio* are of significance to humans: *Vibrio cholerae* is the cause of cholera while *Vibrio parahaemolyticus* and *Vibrio valnificus* both act as agents of acute enteritis or bacterial diarrhea. Several species of which can cause foodborne infection are usually associated with eating undercooked seafood.

In this context, innovative methods have been developed in technology to address these challenges effectively. Nanotechnology, as a groundbreaking and inventive tool, offers a wide range of applications and significant potential for the conservation of aquaculture. It can introduce new methods for drug management and delivery of vaccines, thereby ensuring effective protection for farmed fish against disease-causing pathogens.

The aim of this research was to biologically synthesize silver nanoparticles (AgNPs) using *Hibiscus rosa sinensis* and evaluate the antibacterial activity on *Vibrio* spp. isolated from pathogenic catfish.

Materials and Methods

Collection of Leaf Samples and Extraction of Leaf Extracts

Hibiscus rosa sinensis were aseptically collected from Federal University of Technology, Owerri premises and taken to the laboratory to prepare the leaf extracts using the method described by Naseer *et al*. (2020) as follows; The fresh Hibiscus leaves were thoroughly washed with tap water followed by distilled water to remove contamination and the leaves were air-dried for one week at room temperature ($27^{O}C$) and blended to powdered form. 10g of the powdered leaves were mixed in 500ml of distilled water and heated in a water bath at $70^{O}C$ for 30 minutes, firstly filtered with muslin cloth and finally using Whatman filter paper No. 1, stirred with magnetic stirrer and then centrifuged at 1200rpm thrice for 5 minutes to remove the impurities and heavy biomaterials.

Biosynthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were biologically synthesized from the *Hibiscus rosa sinensis* as described by Jain *et al*., 2009. For 0.1M, 0.5g of silver nitrate was dissolved in 30ml of distilled water and 10ml of the leaf extract was added (i.e. 3:1) while for 0.5M, 2.5g of silver nitrate was dissolved in 30ml of distilled water and 10ml of the leaf extract was added (i.e. 3:1). After which the mixtures were continuously stirred with magnetic stirrer for 15 minutes which led to colour change. Then the mixtures were centrifuged at 10,000rpm for 10 minutes thrice, the supernatants were discarded to remove impurities and the pellets which are the nanoparticles were dried in hot-air oven and stored for further use.

Characterization of Biosynthesized Silver Nanoparticles

Scanning Electron Microscopy Analysis: The synthesized AgNPs were subjected to SEM analysis to determine the surface morphology (Baskaran *et al*., 2018). The sample was made to become thin films and the smallest quantity was poured on the grid which is made of carbon-coated copper (Krithiga et al., 2015). The grid was placed with the sample which was dried for analysis.

UV-Visible Spectrophotometry:

The AgNPs formation was observed through the UV-Visible spectrophotometer. UV-Visible spectrophotometer has a quartz cuvette which has a path length of 1cm. The AgNP sample was placed in the cuvette and the UV-Visible spectrum was produced between 300-700nm wavelength range (Ndikau *et al*., 2017).

Fourier Transform Infra-Red Analysis: The secondary metabolites present in the plant extracts and the functional groups of the AgNPs were identified by the FTIR analysis (Senthil Kumar *et al*., 2006). The stabilization and reduction process of the AgNPs synthesis is carried out by the different functional groups present in the sample.

Collection of Fish Sample

Four live pathogenic catfish samples suffering from skin decolourization, haemorrhages and sluggishness were aseptically collected from a private fish farm at Ohaji/Egbema, Imo State, packed into a sterile ice cooler and transported to Antonie Van Leeuwenhoek Research Centre for isolation of *Vibrio* species.

Isolation of Microorganism

The liver of the diseased fishes aseptically collected using sterile dissecting scissors and scalpel blade. About 5g of each sample was weighed out, homogenized and dispensed into sterile Bijoux bottles containing 50ml of sterile distilled water to serve as stock solution. Ten-fold serial dilution of the stock solution was done and labelled. An aliquot, 0.1ml of 10^{-5} and 10^{-3} dilution tube inoculant were assayed on Nutrient agar and Thiosulfate Citrate Bile Salt agar respectively and spread with sterile glass spreaders on the surface of various solidified sterile agar plates and incubated at 24-48 hours for 37^{O}C. After which the *Vibrio* spp. colonies were isolated (Tsado *et al*., 2013).

Identification of Test Organism

The district and discrete colonies on the different media were isolated and purified by repeated subculturing. The *Vibrio* species isolates were identified using its colonial and morphological characteristics on Thiosulphate Citrate Bile Salt agar and by carrying out various Biochemical tests such as Citrate test, Catalase test, Methyl-red test, Voges-Proskauer test, Indole test, Motility test, Endospore staining test and Gram-staining test as described by Cheesebrough (2003).

Antibacterial Activity of the Synthesized AgNPs

The antibacterial activity of the nanoparticle was determined by well-in-agar diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) as follows; Mueller Hinton Agar was prepared, the nanoparticles at their two different molar concentrations (0.1M and 0.5M) were prepared at various dosages (500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml) by a two-fold serial dilution. Then 1ml of the standardized isolates were seeded on the MHA surface with sterile swab sticks and allowed to solidify. Wells of uniform diameters were created on the agar and sterile pipettes were used to transfer the various dosages of the nanoparticle into the holes using Gentamicin as control. It was allowed to solidify and incubated for 24 h at 37^{O}C. After which the zones of inhibition were measured and recorded.

Bactericidal Activity of the Synthesized AgNPs

The bactericidal effect of AgNPs against *Vibrio* species was studied. The standardized isolates were spread on the surface of Nutrient agar plates

by spread plate method. The two different concentrations of the AgNPs (i.e. 0.1M and 0.5M) at their varying dosages (500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml) were added. After which the plates were incubated for 24 h at 37^{O}C and the growth levels were observed, recorded and the Minimum Bactericidal Concentration was determined.

Results and Discussion

Characterization of Silver Nanoparticles (AgNPs)

The silver nanoparticles biologically synthesized from *Hibiscus rosa sinensis* was confirmed by the presence of colour change from light yellow to dark brown during the synthesis process. Figure 1 showed the corresponding peak in the UV-Visible spectrophotometry at the wavelength of 416.8nm which further confirms the presence of silver nanoparticle formation in correspondence with the work done by Krishnaraj *et al*., 2010 showing the silver to be of pure product and fewer impurities.

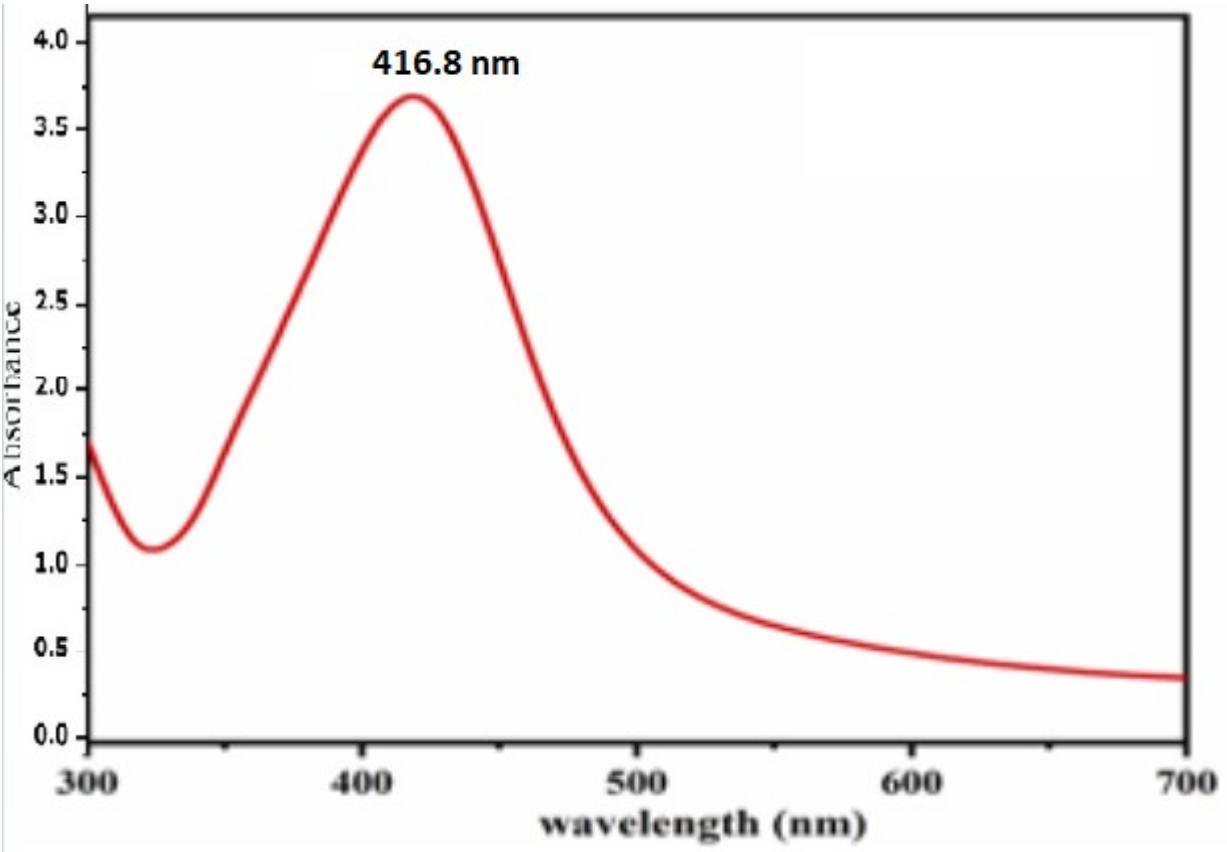

Fig. 1 UV-Vis spectra for silver nanoparticle from Hibiscus plant leaves extract

The nanoparticle behaviour, morphology and shape were ascertained by an accurate measurement using the SEM analysis. SEM analysis revealed that the synthesized silver nanoparticle from the Hibiscus leaf extract exhibited flake-type shapes in aggregated form (Fig. 2). The aggregation may be caused due to polarity and electrostatic attraction of the silver nanoparticles (Vijayakumar *et al*., 2016).

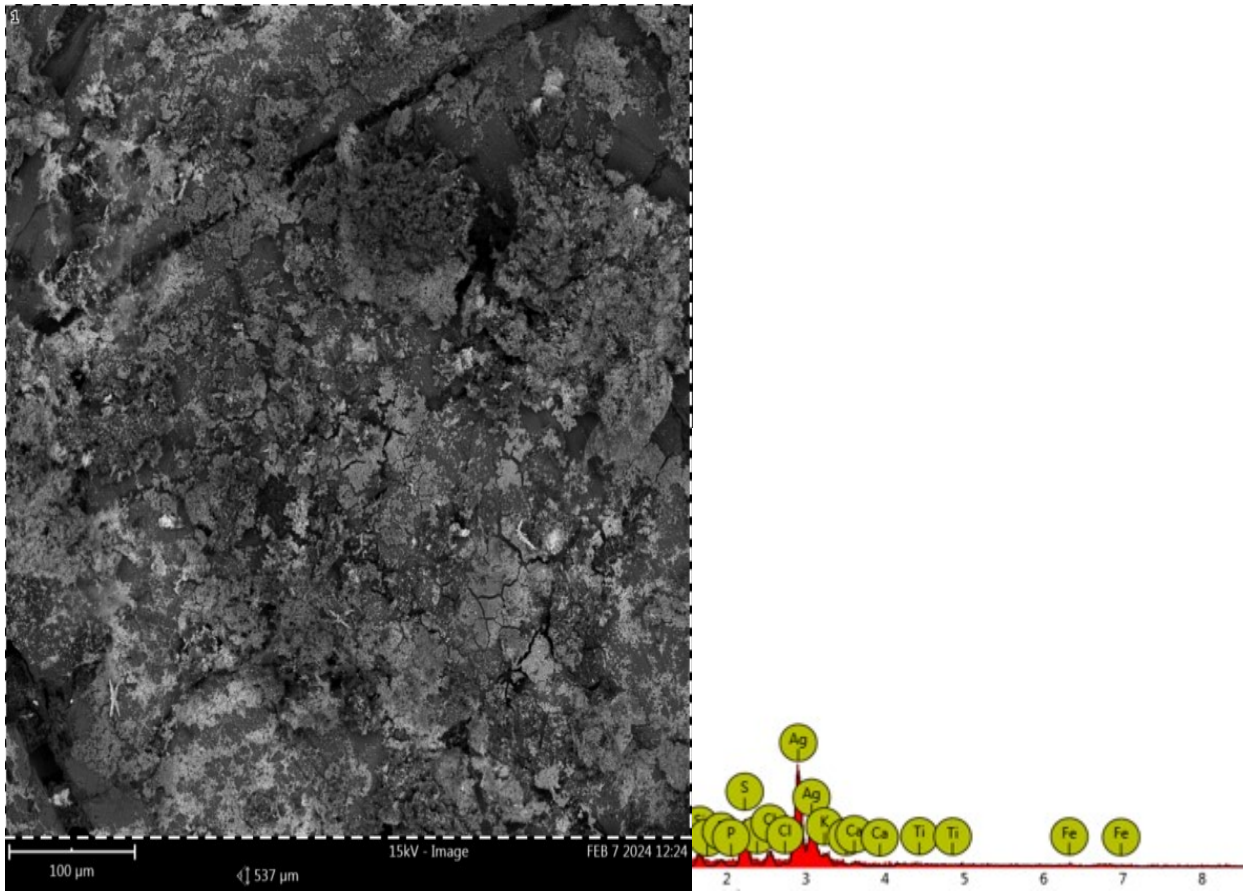

Fig. 2 SEM-EDX for Ag nanoparticle Hibiscus leaves

The FTIR bands (Fig. 3) were expressed in the synthesized AgNPs as follows, 1092.1cm^{-1} showing the stretch of alkyl halides phytochemical group, peak at 1263.6cm^{-1} showed the stretch in aromatic primary amine (C-N) phytochemical group (Sharif *et al*., 2020), 1412.7cm^{-1} exhibit 0-H functional group of bending alcohol as its frequency is between 1410 – 1310cm^{-1} (Hemmalakshmi *et al*., 2017), 1520.8cm^{-1} represented N-H which was a stretch in nitro compound functional group of amides which was in the range of 1550 – 1500cm^{-1} (Sharif *et al*., 2020) while the band 1636.61cm^{-1} represents C = C unsaturated compound alkene functional group showing a stretching vibration in the ketone functional group and phytochemical compound (Sharif *et al*., 2020) and 3287.5cm^{-1} is related to O-H stretch with co-adsorbed H_20 on the Ag surfaces (Li *et al*., 2019; Sharif *et al*., 2020). These peaks may be due to the presence of various phyto constituents expressed during the synthesis namely phenols, alcohols, aromatic compounds, esters and carboxylic groups.

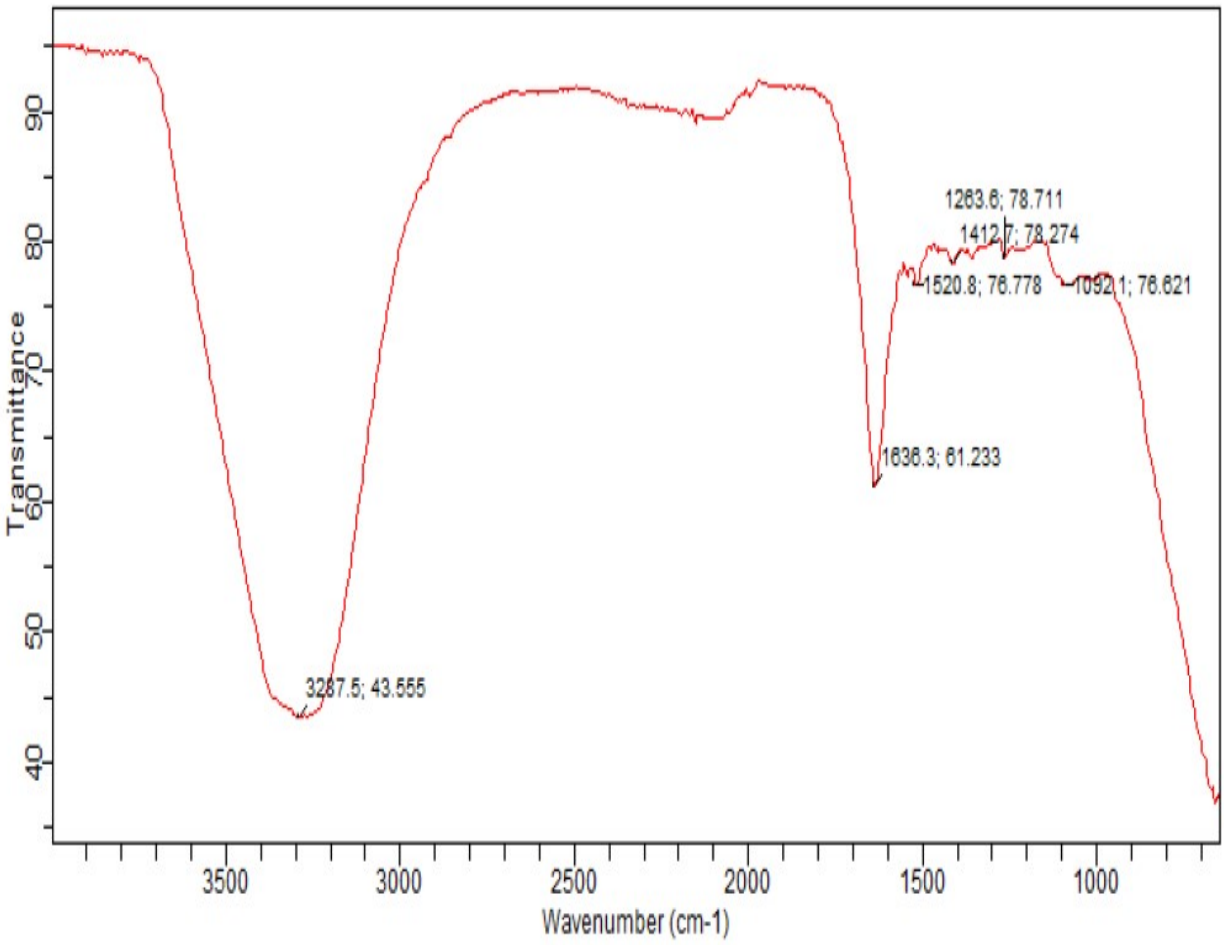

Fig. 3 FTIR spectra for Hibiscus Leaves Silver Nanoparticle

Bacteriological Examination

Results from the culture characteristics of bacteria isolates gotten from the pathogenic fishes cultured on Thiosulphate citrate bile salt agar indicated the exhibition of the typical *Vibrio*species morphological and physiological characteristics, such as flat yellow Gram-negative, motile and non-spore forming rods. Further characterization using biochemical tests which indicated them to be catalase positive, indole positive, citrate positive, methyl-red negative and Voges-Proskauer positive proved these bacteria to be of*Vibrio* spp.

Antibacterial Activity

This present study also focuses on the formation of AgNps and its antibacterial activity against *Vibrio* spp. Pathogens (Fig. 4). The Hibiscus leaf extract and synthesized AgNps showed very good antibacterial activity against the pathogenic microorganism of *Vibrio* spp. which is responsible for causing severe disease outbreaks in aquaculture farms.The *in-vitro* antibacterial activity performed by well-in-agar diffusion method revealed that it was found to be effective in controlling the pathogen. The zone of inhibition was found to be significantly high in the AgNps loaded well of lower molar concentrations (i.e. 0.1M) than the ones at higher molar concentrations (i.e. 0.5M), indicating their enhanced ability to inhibit bacterial growth at lower concentrations, and corroborating the findings of similar studies (Kumar *et al*., 2020; Zhu *et al*., 2021). While the antibiotic control (Gentamicin) generally exhibited higher overall antibacterial activity.

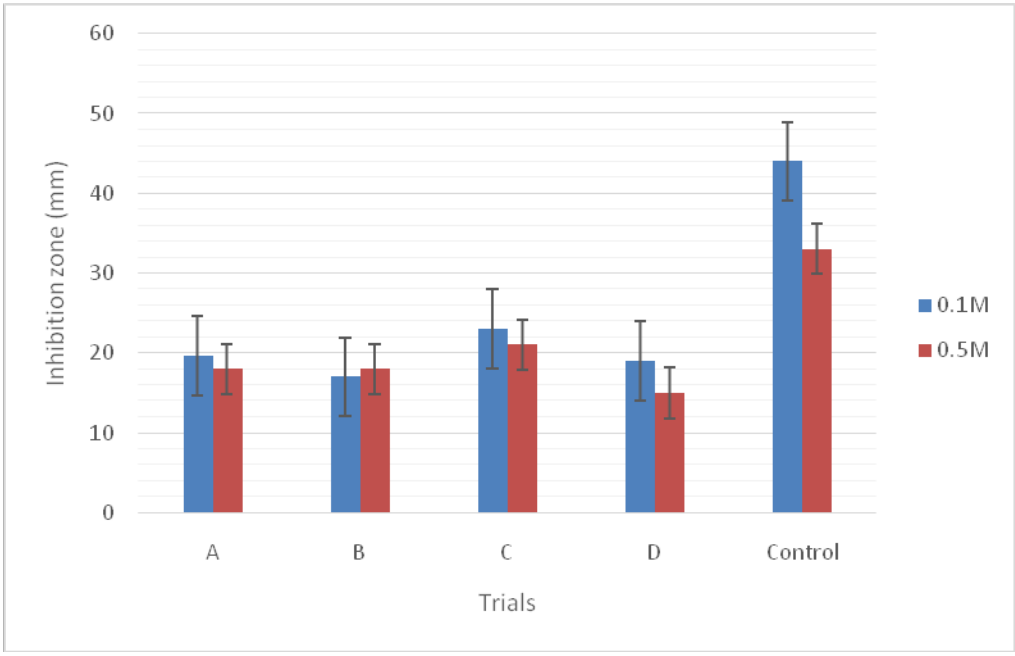

Fig. 4 Mean antibacterial activity of three independent experiments of Silver nanoparticles (AgNps) from 0.1M and 0.5M of *Hibiscus rosasinesis* (Hibiscus leaves) at different concentrations on *Vibrio* sp.
Key: A: 500 mg/ml, B: 250 mg/ml, C: 125 mg/ml, D: 62.5 mg/ml, Control: 80 mg/2ml Gentamicin

Bactericidal Activity

Table 1: Minimum Bactericidal Concentration (MBC) of Silver nanoparticles (AgNps) from 0.1M and 0.5M of *Hibiscus rosasinesis*(Hibiscus leaves) at different concentrations.

Isolates	Hibiscus (0.1M)				Hibiscus (0.5M)			
	A	B	C	D	A	B	C	D
Vibrio spp.	++	+++	+	_*	_*	+	++	+++

KEY:
- = No growth, + = Scanty growth, ++ = Moderate growth, +++ = Copious growth, *= Minimum Bactericidal Concentration, A = 500mg/ml, B = 250mg/ml, C = 125mg/ml, D = 62.5mg/ml

The lowest concentrations with the lowest quantity of growth on the nutrient agar plates at the two different concentrations indicated the efficacy of the nanoparticles to kill the pathogen (Table 1). The lowest dosage (62.5mg/ml) of the lower concentration (0.1M) was the MBC for 0.1M while the highest dosage (500mg/ml) of the 0.5m concentration had the highest bactericidal effect for 0.5m concentration and the bactericidal effect of 0.5M concentration reduced as the dosage reduced suggesting that such variation underscore the importance of considering the potential differences in their modes of action when evaluating the efficacy of nanoparticles as potential antibacterial agents as highlighted by Rajendran *et al*., (2022) and Singh *et al*., (2023).

Conclusion

The findings of this study have demonstrated the potential of silver nanoparticles biologically synthesized from Hibiscus leaves to be effective antibacterial agents against bacterial fish pathogens of *Vibrio* spp. Also, the plant-based origin of the nanoparticle has added benefits as they are derived from eco-friendly and renewable sources which aligns to the principles of sustainable aquaculture practices. It is also essential to acknowledge that the efficacy of this nanoparticle did not surpass that of Gentamicin which was used as the control antibiotic for the study. Several factors such as lack of mechanistic studies, cytotoxicity, suboptimal dosage and concentration may have contributed to this outcome. These findings open up new possibilities to create an awareness on utilization of plant-based nanoparticles as biomedical therapeutics for treatment of bacterial fish pathogens and limit the use of conventional antibiotics in order to reduce antibiotic resistance.

References

Budiati, T., Rusul G., & Wan-Abdullah, W.N. (2013). Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture, 372–375,*127–132.

Rajendran, K., Sen, S., Sinha, C., Ghosh, M., Jena, B. & Mandal, B. (2022). Plant-mediated green synthesis of silver nanoparticles for sustainable aquaculture. *International Journal of Biological Macromolecules, 197*, 375-387.

Singh, S.K., Garg, D., Kumar, P., Ramamurthy, C.H., & Ghosh, S.K. (2023). Plant mediated synthesis of nanoparticles and their application in aquaculture. *Aquaculture, 559*, 738686.

Kumar, P., Nagarajan, A. &Uchil, P.D. (2020). Synthesis of colloidal silver nanoparticles from plants. *Biomimetics for Biomaterials*, 271-315.

Li, B., Han, L., Cao, B., Yang, X., Zhu, X., Yang, B., Zhao, H., &Qiao, W. (2019). Use of magnoflorine-phospholipid complex to permeate blood-brain barrier and treat depression in the CUMS animal model. *Drug Delivery, 26*, 566-574.

Sharif, M., Ansari, F., Malik, A., Ali, Q., Hasan Z., & Khan N.U.H. (2020). Fourier-Transform Infrared Spectroscopy, Antioxidant, Phytochemical and Antibacterial Screening of N-Hexane Extracts of *Punica granatum*, A Medicinal Plant. *Genetics and Molecular Research19* (4), 16039989.

Zhu, Y., Xie, W., Yang, J., Li, Z., Li, Z., Liu, H., Zhang, C., & Xu, D. (2021). Biogenic nanomaterials for sustainable aquaculture: synthesis, application, and prospects. *Environmental Science: Nano, 8*(4), 936-968.

Hemmalakshmi, S., Priyanga, S., & Devaki, K. (2017). Fourier Transform Infra-Red spectroscopy analysis of *Erythrina variegata* L. *Journal of Pharmaceutical Sciences and Research*, *9*(11), 2062-2067.

Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T., & Mohan, N. (2010). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B Biointerfaces*, *76*, 50–56.

Vijayakumar, S., Vaseenharan, B., Malaikozhundan, B., &Shobiya, M. (2016). *Laurus nobilis* leaf extract mediated green synthesis of ZnO nanoparticles: characterization and biomedical applications. *Biomedicine and Pharmacotherapy, 84*, 1213-1222.

Ndikau M., Noah N. M., Andalaand D.M., &Masika, E. (2017). Green synthesis and characterization of Silver nanoparticles using Citrullus lanatus Fruit rind Extract", *International Journal of Analytical Chemistry*, Vol. 2017, Article ID 8108504.

Naseer, M., Aslam, U., Khalid, B., & Chen, B. (2020). Green route to synthesize zinc oxide nanoparticles using leaf extracts of

Cassia fistula and *Melia azedarach* and their antibacterial potential. *Scientific Reports, 10*, 9055.

Abdel-Tawwab, M., Ahmad, M.H., Seden, E.A., & Saleh, F.M. (2010). Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society, 41*, 203-213.

Tsado, E.K., Adesina, O.A., & Oyeleke, S.B. (2013). A survey on the bacterial load of selected fruits and vegetables in Minna metropolis of Niger state. *Journal of Animal Production Advances, 3*(1), 6-11.

Cheesebrough, (2003). District laboratory practice in tropical countries. Part 1, Cambridge University Press. *New York 2003, Second Edition*. 194-197.

Jain, D., Daima, H.K., Kacggwaga, S., & Kothari, S.L. (2009). Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Digest Journal of Nanomaterials and Biostructures, 4*(3), 557-563.

Senthil Kumar, M., Sripriya, R., Vijaya Raghavan, H., &Seghal, P. (2006). Wound healing potential of *Cassia fistula* on infected Albino rat model. *Journal of Surgical Research, 131*(2), 283-2 89.

Baskaran, B., Muthukumarasamy, A., &Maruthai, J. (2018). Biological fabrication of silver nanomaterials and their applications in pharmaceutical fields. *International Journal of Computational Materials Science and Surface Engineering, 10*(2), 79-88.

Krithiga, N., Rajalakshmi, A., &Jayachitra, A. (2015). Green synthesis of silver nanoparticles using leaf extracts of *Clitoriaternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens. *Journal of Nanoscience*, 8.

Access this Article in Online

Website: www.ijcrcps.com

Subject: Nanotechnology

Quick Response Code

DOI: 10.22192/ijcrcps.2024.11.11.001

How to cite this article:

Asonye, C M.,Ogbulie, J.N., Chinakwe, E. C and Braide, W. (2024). Biosynthesis of silver nanoparticles from *Hibiscus rosasinensis* and their antibacterial activities on *Vibrio* species isolated from pathogenic catfish. Int. J. Curr. Res. Chem. Pharm. Sci. 11(11): 1-9.

DOI: http://dx.doi.org/10.22192/ijcrcps.2024.11.11.001

