iJRASET
International Journal For Research in
Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH
IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 Issue: X Month of publication: October 2021

DOI: https://doi.org/10.22214/ijraset.2021.37915

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 9 Issue X Oct 2021- Available at www.ijraset.com

Synthesis of Silver Nanoparticles Using the Leaves of E*ichhornia Crassipes* and its Effect on Fish Infecting Pathogens

Dr. M. Nithiyasoundari[1], Dr. Seeli Balaji[2], Dr. K. S. Parimala[3]
[1]*Assistant Professor in PG Department of Microbiology, New Prince Shri Bhavani Arts and Science College,Medavakkam,Chennai-100*

Abstract: Owing to the increasing importance of aquaculture to replace the progressive reduction of farmed fish due to the increase of bacterial infections. The present study aimed to develop more environmental friendly biological compound to control the bacterial infections. The antibacterial activity was performed with aqueous plant extract and the silver nanoparticles from the leaves of Eichhornia crassipes. The characterization of the silver nanoparticles were done by using UV-Spectrophotometer, FTIR and TEM. Fish infecting pathogens were isolated and taken from various parts of the infected C.batrachus. In this study, the bacterial strains of A.hydrophila, P.aeruginosa, V.cholerae and E.tarda were used. The antibacterial activity was done with the use of Agar well Diffusion method.
Keywords: Aqueous plant extract, silver nanoparticles, UV, FTIR, TEM and Antibacterial activity

I. INTRODUCTION

Cultured fishes are constantly threatened by microbial attacks. The main biological agents that cause water-borne diseases are bacteria, viruses, protists and helminths, oomycetes and, to a lesser extent, fungi. However, bacterial diseases are main problems in the expanding aquaculture industry [1-3].

Several fish farming plants often suffer from heavy losses owing to the frequent development of infections caused essentially by bacteria. There are two broad groups of bacteria of public health significance that contaminate fish: those naturally present in the environment-the indigenous microflora (e.g., *Photobacterium damselae*, *Vibrio anguillarum*, *V. vuln ificus*, *Aeromonas hydrophila*, *Aeromonas salmonicida*) and those introduced through environmental contamination by domestic animals excreta and/or human wastes – non-indigenous microflora (e.g., Enterobactereaceae such as *Salmonella sp.* and *Escherichia coli*) [4-10].

In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials [11]. Nanotechnology is a field that is mushrooming, making an impact in all spheres of human life. A number of approaches are available for the synthesis of silver nanoparticles viz, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route [12].

Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [13]. The most widely used and known applications of silver and silver nanoparticles are in the medical industry. These include topical ointments and creams containing silver to prevent infection of burns and open wounds [14].

Water hyacinth, *Eichhornia crassipes* also known as 'blue devil', grows rapidly as a dense green mat over stagnant water bodies such as lakes, streams, ponds, waterways, ditches and backwaters. It has been considered as an uncontrolled nuisance many a time. The capacity of water hyacinth to invade and overtake aquatic habitats is astounding. It can quickly dominate natural areas and can dramatically alter the species composition, structure and function of native plant and animal communities. It alters the ecosystem of the water body, causing dissolved oxygen fluctuations and raising the water temperature [15-16].

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Herbal remedies and alternative medicines are used throughout the world and in the past, herbs often represented the original sources of most drugs [17]. Most important of these bioactive constituents of *Eicchornia crassipes* leaves of the plants are alkaloid, tannins, sterols, anthroquinones, flavonoids, phenolic compounds and proteins [18].

Plants contains flavonoids, alkaloids, Tannins, phenols etc., which have biological significance in terms of medicine development and extracts of aqueous, methanol and ethanol are good source of antiviral, antitumor and antibacterial agents [19]. The bioactive compounds act as self defence against pests and pathogens [20].

In this way, to reduce the risk of development and spreading of microbial resistances and to control fish diseases in aquaculture, alternative strategies must be developed to allow the use of reasonably cheap and more environmentally friendly methods. In this present study investigate the synthesized silver nanoparticles were used to inactivate the microorganisms in fish farming plants as well as the new environmentally friendly approaches to control fish infection.

II. MATERIALS AND METHODS

A. *Collection of plant*

The fresh aquatic leaves of *Eichhornia crassipes* were collected from Pallavaram Lake, Tamil Nadu, and India. The voucher specimen was submitted to Prof.P.Jayaraman, Ph.D. Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, and Chennai for taxonomic identification of the plant.

The leaves were washed thoroughly in running tap water and finally washed with sterile distilled water. The leaves were shade dried and ground into coarse powder. Twenty five grams of powdered sample was extracted with 200 ml distilled water for 24 hrs using Soxhlet apparatus and filtered through what mann filter paper .The filtrate was then subjected to Vacuum evaporator for getting the crude extract.

B. *Collection of culture*

Cultures were collected from IMTECH Laboratory (Institute of Microbial Technology, Chandigarh, India). These organisms were maintained on Nutrient agar slants and stored for the future experiments.

C. *Synthesis of Silver Nanoparticles*

25g of Leaves of *Eichhornia crassipes were* taken and mixed with 200ml of deionised water and kept it for boiling for about 25minutes. The aqueous medium was filtered through Whatmann filter paper and the filtrate was used for the further experiments. 10ml of aqueous leaf extract was mixed with 90 ml of 1mM silver nitrate solution and kept it for sunlight for 20 minutes. The reduction of Ag^+ ion in the aqueous medium was confirmed by the color change from yellow to brown.

D. *Characterisation of Nanoparticles*

1) *UV-Vis Spectrophotometer:* The reduction of Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using DU 800 Spectrophotometer.
2) *FTIR:* FTIR spectra were obtained using a Bruker Spectrum 100 spectrophotometer, operated at the resolution of 4 cm-1. The sample was drop cased on a silicon wafer and the material was analysed and the spectra was recorded in diffuse reflectance mode.
3) *TEM:* Sample for transmission electron microscopy (TEM) was made by drop casting the silver nanoparticles solution onto a carbon coated copper grid and performed using a JEOL 1010 TEM instrument operated at an accelerating voltage of 100 kV.

E. *Agar-Well diffusion Method*

The agar well diffusion method was performed for determining the antibacterial activity of crude aqueous leaf extract of *E.crassipes* and the Synthesized silver nanoparticles from leaves of *Eichhornia crassipes* against all the selected bacterial isolates of *P.aeruginosa, A.hydrophila, Vibrio cholerae, E.tarda.* Antibacterial activity was obtained by determining the zone ofinhibition around the well.

III. RESULT AND DISCUSSION

A. *UV-Vis Spectrocsopy*

The bioreduction of Ag^+in aqueous solution was monitored by UV-vis spectroscopy. The absorbance spectra of synthesised nanoparticles were detected at various absorbance 400nm to 800nm.The synthesised silver nanoparticles from the aqueous solution found at the absorption peak around 550nm that indicates the particles are completely dispersed in the aqueous solution.

Visual color change from pale yellow to brown when *Talinum fruiticosum* leaf extract added dropwise to silvernitrate was the primary evidence for the formation of silver nanoparticles. The silver nanoparticles formed by the reduction of Ag+ into Ag0. The reduction reaction is facilitated by the extract of *Talinum fruiticosum.* The appearance of brown color in aqueous solution is due to excitation of surface plasmon vibrations. It is observed that the surface plasmon resonance of silver nanoparticles is between 200 - 450nm [21].As the concentration of the *Talinum fruiticosum* leaf extract increases,the absorption peaks gets more sharpness and blue shift was noted which indicates a reduction in the size of silver nanoparticles. This result was in correlation with the report of green synthesis of silver nanoparticles by *Aegle marmelos* leaf extract [22].

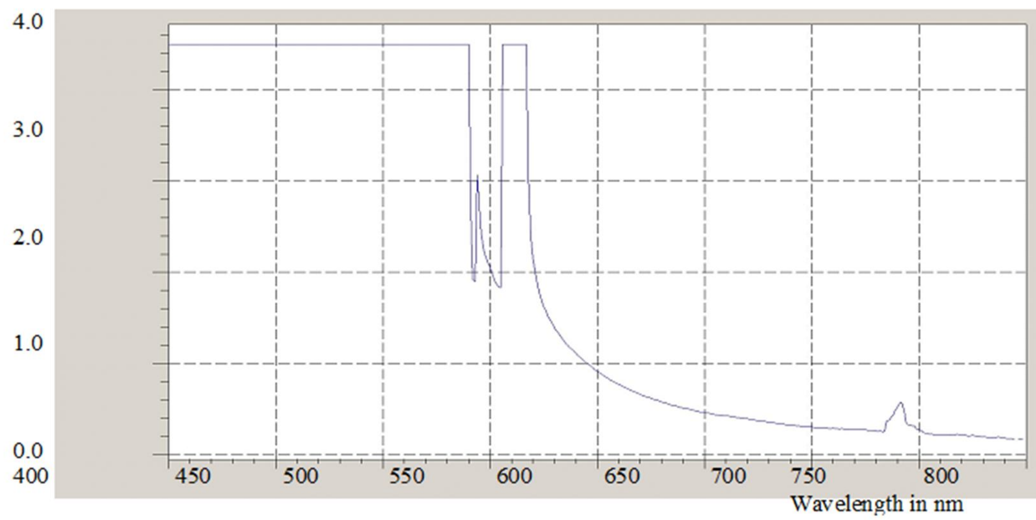

Fig-1: Reduction of Ag ions in aqueous extract of *E.crassipes*

B. FTIR

FT-IR spectrum was used to identify and recorded the possible phytochemical in *E.crassipes* leaf extract responsible for capping lead to efficient stabilisation of silver nanoparticles. The absorption at 3377, 2926 cm-1 correspond to the O-H stretching vibrations of phenols. Peaks correspond to the wave numbers 1602, 1384, 1117, 1087 cm-1 show the presence of O-H stretching of alcohol/ phenol. The absorption peak between 917cm-1 & 617 cm-1 is responsible for C-H out of plane bending of aromatic hydrocarbons. These results confirm the presence of poly phenolic groups responsible of capping and stabilization of silver nanoparticles.

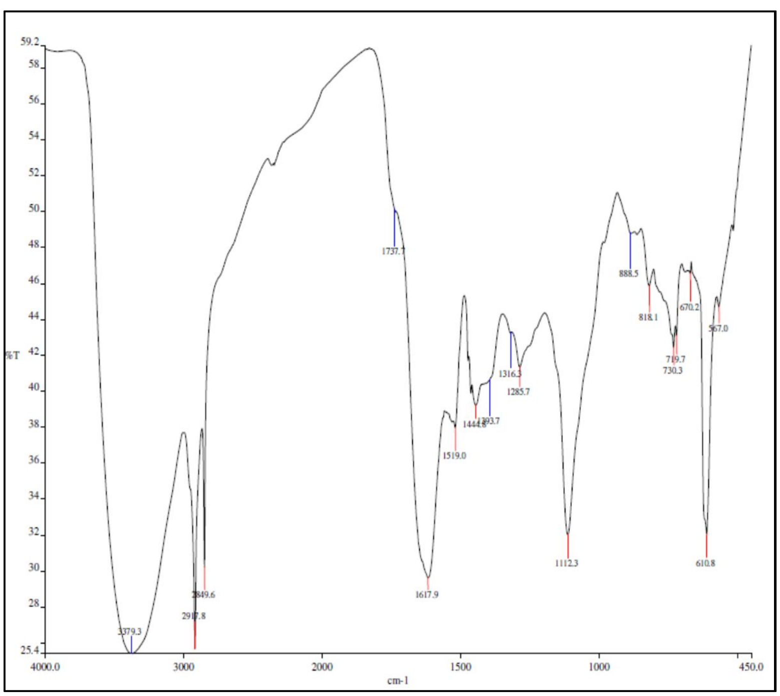

Fig- 2: Analysis of phytochemicals in *E.crassipes* leaf extract

C. TEM

The size and morphology of nanoparticles were evaluated by TEM analysis. The TEM image shows the spherical shape of the silver nanoparticles. The nanoparticles are quite polydisperse and ranged in size from 40 to 50 nm with an average size of ca. 12 nm. Generally, the nanoparticles are well dispersed, although some of them were noted to be agglomerated. The TEM images clearly reveal the presence of some organic layer surrounding the surface of the AgNPs. Notably, the majority of the particles in the TEM images are not in physical contact with each other but appeared separated by the organic layer. Therefore, high-resolution TEM images clearly indicate the coating of AgNPs with an organic layer [23].

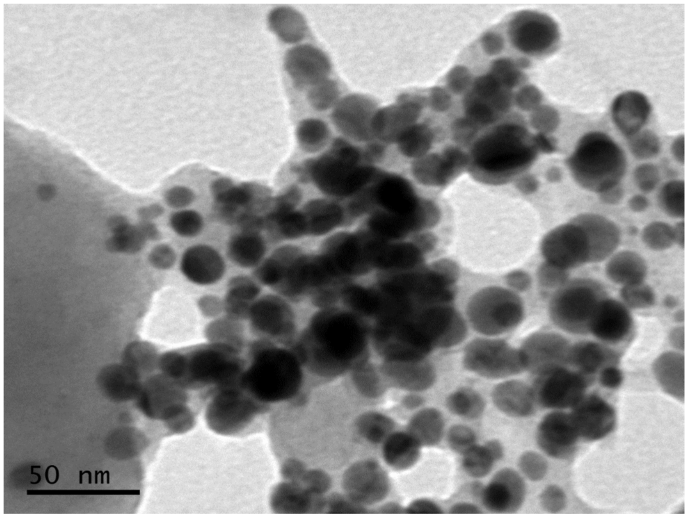

Fig- 3: Analysis of silver Nanoparticles using the leaves of *E.crassipes*

D. Antibacterial Activity

The antibacterial activity was performed for the aqueous plant extract and the silver nanoparticles against the selected bacterial fish pathogens. *A.hydrophila* has showed more activity in synthesized silver nanoparticles (15mm) than the aqueous plant extract (13mm).In *P.aeruginosa* also showed more activity in synthesized silver nanoparticles (12mm) than the plant extract (11mm). *V.cholerae* showed minimum antibacterial activity in plant extract and synthesized silver nanoparticles respectively (3mm, 5mm). *E.tarda* has showed only activity in plant extract (8mm) and there is no activity showed in silver nanoparticles.

Euphorbia hirta aqueous leaf extract was used for biosynthesizing AuNPs. NPs sizes ranged from 6–71 nm, which greatly contributed to their highly effective antimicrobial activity against bacterial strains of *E. coli*, *P. aeruginosa*, and *K. pneumonia*. The study evaluated concentrations of 1.25–200 µg/ml, where 200µg/ml completely inhibited the bacterial growth [24].

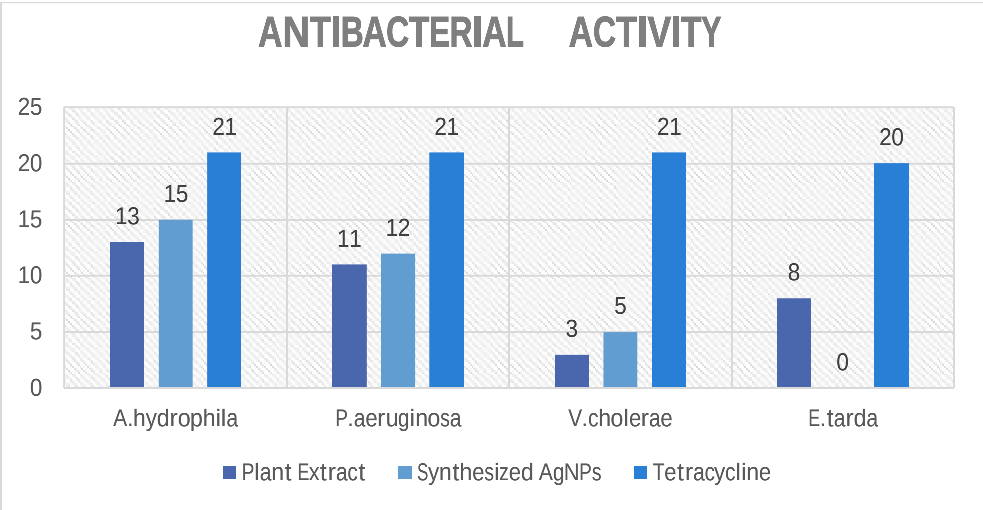

Fig-4 Antibacterial activity against selected bacterial pathogens

IV. CONCLUSION

The novel approach used to inactivate fish pathogenic microorganisms with synthesized silver nanoparticles using the leaves of E.carssipes is outstanding, safety to the fishes and aquaculture environment. In this study we have proved that the aqueous leaf extract of *E.crassipes* is suitable for the synthesis of silver nanoparticles. The aqueous plant extract and the synthesized silver nanoparticles were showed the effective antibacterial activity against the fish infecting microorganisms. From this study the plant showed that they have more potential applications in aquaculture, pharmaceutical and biomedical fields.

V. ACKNOWLEDGEMENT

Authors are thankful to Dr.selvakannan, Research fellow, RMITY University, Melbourne, Australia and Shankara Nethralaya, Laboratory, Chennai, Tamilnadu and India for provided the Laboratory facility for my work.

REFERENCES

[1] Alderman, D.J. Geographical spread of bacterial and fungal diseases of crustaceans. Rev. Sci. Tech. OIE 1996, 603-632.
[2] Shao, Z.J. Aquaculture pharmaceuticals and biologicals: current perspectives and future possibilities. Adv. Drug Delivery Rev. 2001, 50, 229-243.
[3] Wahli, T.; Knuesel, R.; Bernet, D.; Segner, H.; Pugovkin, D.; Burkhardt-Holm, P.; Escher, M.; Schmidt-Posthaus, H. Proliferative kidney disease in Switzerland: current state of knowledge. J. Fish Dis. 2002, 25, 491-500.
[4] Muroga, K.; Iida, M.; Matsumoto, H.; Nakai, T. Detection of Vibrio anguillarum from waters. Nippon Suisan Gakk. 1986, 52, 641-647.
[5] Muroga, K.; Tanasomwang, V.; Momoyama, K. Vibrio a nguillarum infection in juveniles of tiger puffer (Takifugu rubripes). Fish Pathol. 1987, 22, 29-30.
[6] Nakai, T.; Park, S.C. Bacteriophage therapy of infectious diseases in aquaculture. Res. Microbiol. 2002, 153, 13-18.
[7] Fukuda, Y.; Nguyen, H.D.; Furuhashi, M.; Nakai, T. Mass mortality of cultured sevenband grouper, Epinephelus septemfasciatus, associated with viral nervous necrosis. Fish Pathol. 1996, 31, 165-170.
[8] Iida, H.; Tanaka, S.; Shibata, Y. Small GTP-binding protein, Rab6, is associated with secretory granules in atrial myocytes. Am. J. Physiol. Cell Physiol. 1997, 272, 1594-1601.
[9] Nakai, T.; Sugimoto, R.; Park, K.; Matsuoka, S.; Mori, K.; Nishioka, T.; Maruyama, K. Protective effects of bacteriophage on experimental Lactococcus garvieae infection in yellowtail. Dis. Aquat. Org. 1999, 37, 33-41.
[10] Huss, B.C.R. Gershom Scholem's major trends in Jewish Mysticism: 50 years after. In Proceedings of the Sixth International Conference on the History of Jewish Mysticism, 1994; pp. 1265-1267.
[11] A. J. Gavhane, P. Padmanabhan, S. P. Kamble and S. N. Jangle, Int J Pharm Bio Sci., 2012, 3(3), 88 – 100.
[12] V.Manonmani, Vimala Juliet, International Conference on Innovation, Management and Service, IPEDR, IACSIT Press. Singapore. 14, 2011.
[13] H. Jiang, S. Manolache, A.C.L. Wong, F.S. Denes, J. Appl. Polym Sci. 93 (2004) 1411–1422.
[14] R.O. Becker, Met. Based Drugs 6 (1999) 297–300.
[15] Vilaseca, M., Gutie, M.C., Grimau, V.L., Mesas, M.L. and Crespi, M., 2010. Biological treatment of a textile effluent after electrochemical oxidation of reactive dyes. Water Environ. Res. 82, 176–181.
[16] Awomeso, J.A., Taiwo, A.M. and Gbadebo, A.M., Adenowo, J.A., 2010. Studies on the pollution of water body by textile industry effluents in Lagos, Nigeria. J. Appl. Sci. Environ. Sanit. 5, 353–359.
[17] Sa'ad B., Azaizeh, H. and Said, O. 2005. Traditional and perspectives of Arab herbal medicine: A review, Evid Based complements Alternative Medicine. 2: 475 -479.
[18] Cowan MM. Plant products as antimicrobial agents. Clin Micro Rev 1999; 12(4): 564–582.
[19] Harborne, J.B. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd edition). Chapman and HallCo., New York. Pp.1-302.
[20] Krishnamurthy NB, Nagaraj B, BarasaMalakar, Liny P, and Dinesh R "Green synthesis of gold nanoparticles using tageteserectal(mari gold) flower extract & evaluation of their antimicrobial activities" international journal of pharma and bio sciences , (3): 212-22, (2012).
[21] Sulochana S., Palaniyandi Krishnamoorthy and Sivaranjani K.Journal of Pharmacology and Toxicology Volume 7, Number 5, 219-230, 2012
[22] Khalil MMH , Ismail EH , El-Baghdady KZ , Mohamed D : Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity . Arab J Chem 2013: 1 – 9
[23] Sankar, R.; Karthik, A.; Prabu, A.; Karthik, S.; Shivashangari, K.S.; Ravikumar, V. Origanum vulgare mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. Colloids Surf. B Biointerfaces 2013, 108, 80–84.
[24] Annamalai, A.; Christina, V.L.P.; Sudha, D.; Kalpana, M.; Lakshmi, P.T.V. Green synthesis, characterization and antimicrobial activity of Au NPs using Euphorbia hirta L. leaf extract. Colloids Surf. B Biointerfaces 2013, 108, 60–65.