IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Fabrication and Characterisation of Silver Nanoparticles with *Illicium Verum* Seed Extract and Evaluation of Its Anti-Bacterial Activity

[1]Gobalan K, [2]Sudharsan P, [3]Purushothaman T; [4]Sumithra D; [5]Siva Vijayakumar T*

[1]Assistant Professor, [2]Research Scholar, [3]Assistant Professor, [4]Assistant Professor, [5]Assistant Professor

[1] Department of Biotechnology, Jamal Mohamed College, Trichy, Tamil Nadu, India.

[2] Department of Forestry, Nagaland University (A Central University), Lumami, Nagaland, India.

[3] Department of Biotechnology and Research, SNMV College of Arts and Science, Malumichampatti, Coimbatore, Tamil Nadu, India.

[4] Department of Biotechnology, Bon Secours College for Women (Affiliated with Bharathidasan University), Thanjavur, Tamil Nadu, India.

[5] PG and Research Department of Biotechnology, Srimad Andavan Arts and Science College (Autonomous), Trichy, Tamil Nadu, India.

Abstract: Nanoparticles are frequently used in a variety of scientific and engineering disciplines worldwide. By using particle size, nanoparticles are used to quickly deliver a material to a particular place. The fabricated silver nanoparticles were verified by UV spectroscopy, DLS, FTIR, and SEM studies. The characterizations confirmed that the fabricated material was nanoparticles, and two bacteria species, *S. aureus and E. coli*, were used in the tests to determine silver nanoparticles antibacterial potency. The establishment of anti-bacterial zones demonstrates the ability of the produced nanoparticles to stop the growth of *E. coli* and *S. aureus*. This study concludes that the silver nanoparticles have the ability to inhibit the growth of microorganisms like *E. coli* and *S. aureus*.

Index Terms **– Silver nanoparticles, Antibacterial, SEM, FTIR, DLS, *E. coli, S. aureus.***

INTRODUCTION

Nanotechnology is the branch of science and engineering that deals with how to control and manipulate Nano scale particles. These nanoparticles range from 1 to 100 nm. The word nano comes from the Greek word “Nanos” meaning very small. They have a size of one billionth of a meter. The Nano-particles are grouped in to two types namely organic and inorganic nanoparticles of carbon and magnetic or semiconductor nanoparticle respectively. Apart from these other Nano materials are the polymeric nanoparticle, solid lipid Nanoparticle, liposomes, Nano crystal, Nanotubes, Dendrimers and many more (Heera & Shanmugam, 2015). Nanoparticles can be synthesized by three methods namely physical, chemical and biological methods. In comparison with other 2 methods, biological method is considered as the rapid, safer and cost-effective alternative to physical and chemical methods.

Nanoparticles are classified as carbon based, metal, ceramics, semiconductor nanoparticles, polymeric and lipid-based nanoparticles. The characterization of nanoparticles can be done by different methods like UV-visible absorption spectroscopy, XRD, XPS, IR, SEM, TEM, DLS and FTIR methods. This characterization method is done to analyse the physiochemical properties of nanoparticle. The synthesized nanoparticles are used in wide variety of applications like drugs, medications, development of printed electronics, sensors and many more (Khan et al., 2019). Other applications include the bio-sensing, drug delivery, bioremediation, water treatment and production of clean energy and many more (Tiquia- Arashiro et al., 2016). Plants are the nature's treasure. They are the wealth to the human society. Human beings and animals were dependent on plants for their food, shelter, clothing and as well as medicine (Gurib-Fakim, 2006). Different parts of plant have different medicinal properties. Medicines obtained from plants are the herbal medicines. They have no side effects when compared to chemical drugs. There are about 3,50,000 species of plants (Pan et al., 2014). India is one of the richest sources of medicinal plants. These medicinal plants are known to cure any types of disease like diabetes, gastrointestinal problem and many more. Plants are used as drugs since 60,000 years ago. Around 50% of drugs are the richest source of medicinal plants. So, the track has changed from synthetic drugs to herbal drugs (Jamshidi- Kia et al., 2018). The aim of the study to determine the anti-bacterial activity of silver Nanoparticles synthesized from seed extracts of *Illicium Verum* against *E. coli* and *S. aureus*.

MATERIALS AND METHODS

PREPARATION OF SEED EXTRACT

Illicium Verum seeds were collected and then 1g was taken and dried at room temperature. The seeds were crushed with mortar and pestle into a fine powder. 20 ml of distilled water was taken in a beaker to that 1g of crushed *Illicium Verum* seeds were added and boiled at 60˚C for 10 minutes until bubbles appeared. The extract was cooled and filtered by using whatman filter paper and stored at 4º C (Sudharsan et al., 2022).

Synthesis of silver nanoparticles

9.04 mg of silver nitrate (AgNO3) was dissolved in 53.23 ml of distilled water from that the solution was divided into half for the addition of extract. *Illicium Verum* seed extract of 0.532 µl of extract solution was added to 26.615 ml of AgNO3 and stirred using magnetic stirrer and it was also subjected to heat. The colour change was observed for every half an hour. The extract solution was then subjected to centrifugation and pellet was obtained, to that add water of 2ml in Eppendorf tube to take OD of the extract (Luna et al., 2015).

UV-visible light spectroscopy

The range of UV absorption were taken between 300 to 700 nm. The deionized water was used as a blank. The OD of star anise seed extract synthesized silver nanoparticles was observed for every one hour. The peak was observed at 437 nm. The OD was observed till 8th day to determine the stability of sample. The Eppendorf tube which was centrifuged was also checked for OD (Elamawi et al., 2018).

FT-IR Spectroscopy

Illicium Verum seed extract and Fabricated AgNPs were subjected to FTIR to identified what are all the functional groups present in the nanoparticles. The presence of functional group is identified in the range of 4000-400cm-1. Here the solutions of $AgNO^3$ are dried at 75˚c and dried powder were characterized using KBR pellet method (Damayanti et al., 2020).

DLS (Dynamic Light Scattering Spectroscopy)

DLS is also known as photon-correlation spectroscopy. It is mainly used to determine particles size distribution. DLS is based on laser diffraction method with multiple scattering technique to study average particle size of silver Nanoparticles. When a monochromatic beam of light is passed through a solution, a Doppler shift occurs, thereby changing the wavelength of the incoming beam of light by a value related to particle size (Patra & Baek, 2014).

SEM (Scanning Electron Microscopy)

The morphological features of synthesized silver nanoparticles from seed extract of star anise were studied by scanning electron microscopy (JSM-6480 LV). After 24hrs of the addition of AgNO3 the SEM slides were prepared by making a smear of the solutions on slides (Patra & Baek, 2014). A thin layer of platinum was coated on the samples to make it conductive. They were characterized in SEM at a voltage of 15 KV.

Anti-bacterial activity

25ml of Luria Bertani broth prepared and were inoculated with pure culture of *E. coli* and *S. aureus* and they were kept in incubator (Sri et al., 2015). 150ml of Muller Hinton agar and Agar solution was prepared. The agar solution was poured on each petriplate to get solidified. The culture of *E. coli* and *S. aureus* was spread over the agar using cotton swabs. Holes were made with gel puncher and AgNO3 nanoparticles of different concentrations (25, 50, 75, 100µl) was poured in each well and kept in incubator at 36+/-1℃ for 24 hours to observe the zone of inhibition. Streptomycin was used as standard drug. (Salem et al., 2021).

RESULTS AND DISCUSSION

Synthesized silver nanoparticle analysis

The Star Anise seed extract was kept under room temperature for 2 days and later colour change was observed. The colour of the solution changed from light pale yellow to mild brown colour. The change of colour indicates that silver nanoparticle has been synthesized (Aayasha et al., 2021)

UV-visible spectrophotometer analysis:

Reduction of silver nanoparticles during exposure to seed extracts was observed as a result of the colour change. A visible colour change was observed from pale yellow to mild brown colour within 2 hours. This indicates the formation of silver nanoparticles, after 48 hours, there was a significant colour change from mild to little bit dark brown colour due to increase reaction time, it enhances the growth of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in water due to Surface Plasmon Resonance phenomenon.

The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed at 437nm. In case of seed extract of star anise, the intensity of absorption peak increases with the increase in time period (Figure 1). The metal nanoparticles were observed o be stable in solution even after 3 weeks of their synthesis. By stability we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time. The intensity of absorption peak at 437nm increases with increasing time period of aqueous component.

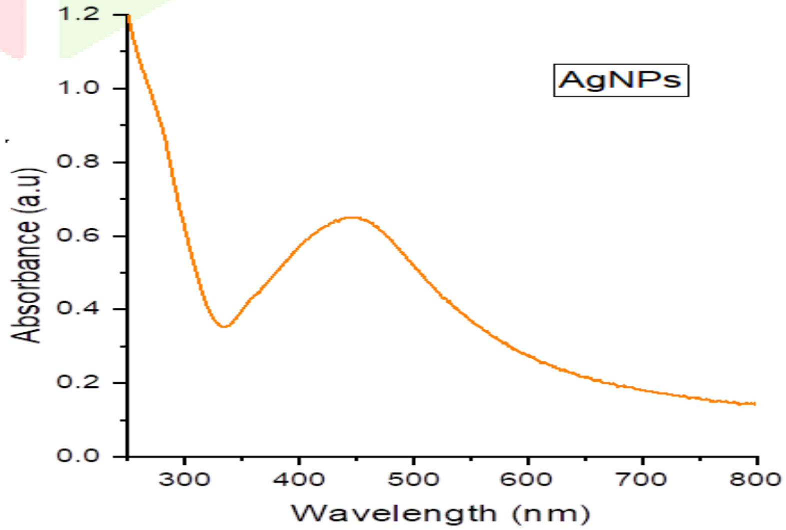

Figure 1: UV peak for synthesized silver nanoparticle

FT-IR Analysis

FT-IR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticle synthesis. The Figure 2 shows the sharp FT-IR spectrum of synthesized AgNPs located at about 665.16cm-1, 1634.21cm-1, 2073.60cm-1, 3435.39cm-1. The absorption peak at 665.16cm-1 showed the stretch of silver nanoparticles. The peak found at 1634.21cm-1 shows the bond stretch of N-H. The peak at 2073.60cm-1 shows C-H bond and the peak corresponding to 3435.39cm-1 shows O-H stretching, H- bonded alcohol and phenols. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups (Alsalhi et al., 2016).

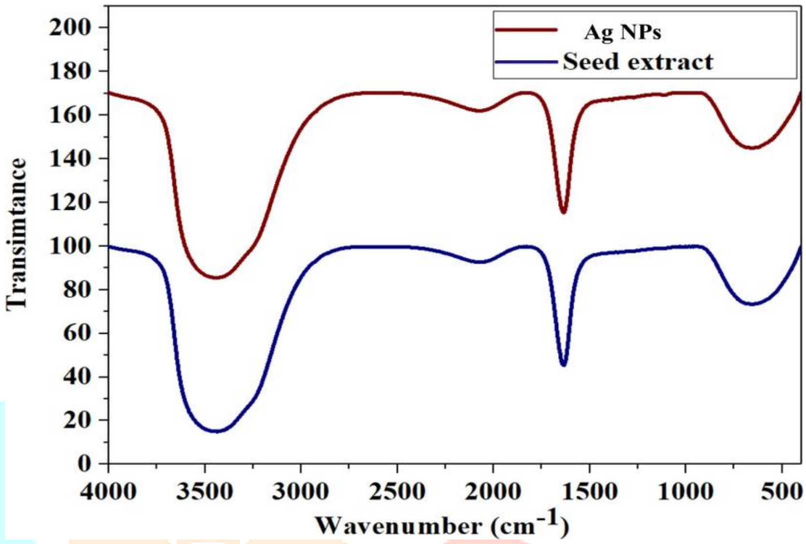

Figure 2: FTIR peak for fabricated Nanoparticles and extract

From the analysis of FT-IR studies, we confirmed that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles capping of silver nanoparticles to prevent agglomeration and thereby stabilize the medium. These results suggest that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

DLS Analysis

DLS technique is a diagnostic tool for to analyze the particle size distribution of silver nanoparticles in solution or colloidal suspensions has been widely used in science and industry. The DLS size distribution of synthesized silver nanoparticles are shown in figure 3. The calculated average particle size distribution of silver nanoparticles is 90 nm. The broad spectrum of DLS analyzer confirms that the particle size is decreased when compared with SPR peak (437nm) obtained in UV spectra. Hence DLS is defined as a technique by changing the scattering light intensity fluctuations to obtain the sample average hydrodynamic diameter. Since the measurement process of DLS is rapid and sensitive solution phase detection, this technique has been applied to detect metal ions and cancer biomarkers.

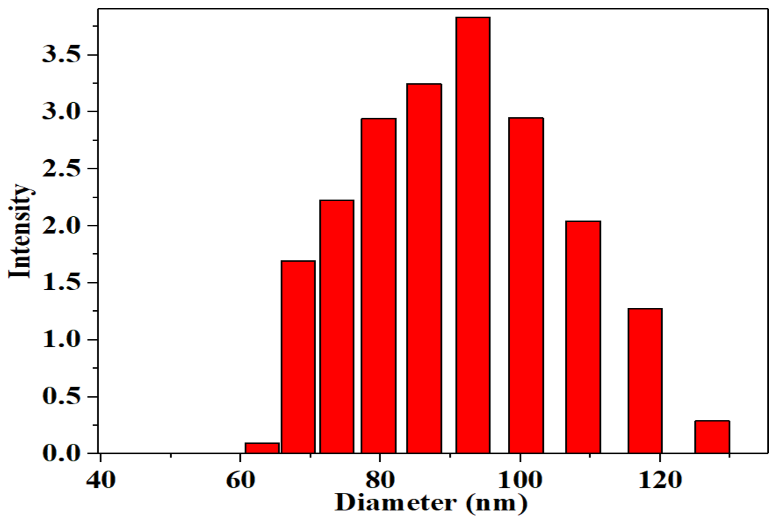

Figure 3: DLS analysis of fabricated nanoparticles

SEM Analysis

SEM is a technique that uses electrons instead of light to form an output image. The SEM analysis is employed to characterize size, shape, morphology and distribution of synthesized silver nanoparticles. The SEM micrographs indicate the purity and poly-dispersity of resulting silver nanoparticles shown in figure 4. SEM images typically showed that silver nanoparticles are predominantly spherical in shape having smooth surface and well dispersed with close compact arrangement. The size of the prepared nanoparticles was more than the size of nanoparticle which should be between 1-100nm but the size ranged from 100-140nm. The size was more than the desired size as a result of proteins which were bound in the surface of the nanoparticles.

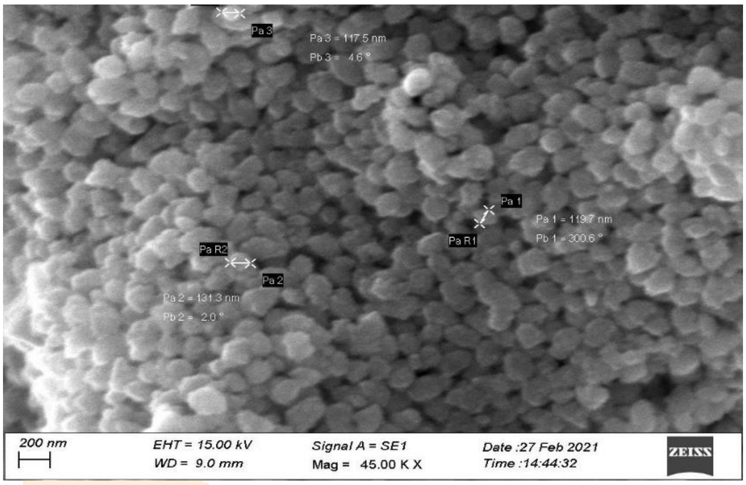

Figure 4: SEM analysis of fabricated Nanoparticles

Anti-Bacterial activity

The images show the anti-bacterial effects of various concentration of star anise seed extract (25, 50, 75, 100 µl). The silver nanoparticles against *S. aureus* (Gram positive bacteria) and *E. coli* (Gram negative bacteria) were studied by agar well diffusion method. Silver nanoparticles showed significant anti-bacterial activity against these pathogens. The inhibition zone formed were silver nanoparticle dose dependent and maximum zone of inhibition was obtained at 100µl. The high anti-bacterial potential of silver nanoparticles may be due to large surface area, shape and ultrafine structure. The synthesized silver nanoparticles help to deactivate cellular enzymes and damage the bacterial cell membrane because of positively charged silver ions. This leads to enzymatic dysfunction and damage to proteins and DNA because of reactive oxygen species by silver nanoparticles ending in cell death. These spherical shaped silver nanoparticles are responsible for the inhibition of gram negative bacterium *E. coli*. Anti-bacterial activity of synthesized silver nanoparticles is observed against both *E. coli* and *S. aureus* at a higher concentration of 75µl and 100µl and a clear zone is also formed and shown in figure 5. Here antibiotic Streptomycin is used as control. These bacterial group incubations around the wall are due to the release of diffusible inhibitory compounds from silver nanoparticles, these biosynthesized silver nanoparticles are used in cancer therapy, wound healing and anti-bacterial activity and also in medical field. The zone of inhibition possesses that silver nanoparticle has effective antibacterial property (Prabhu et al., 2022).

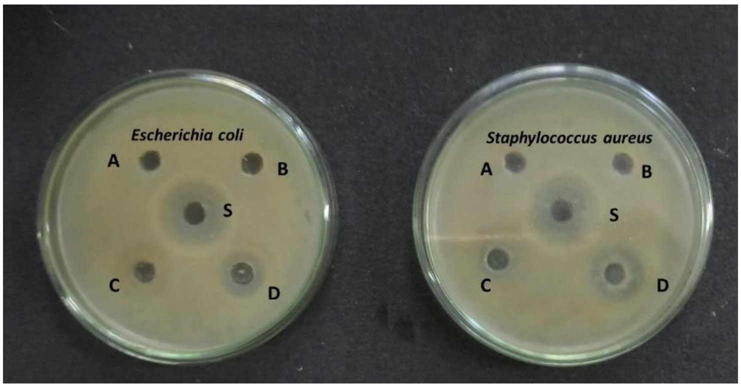

Figure 5: Anti-Bacterial activity of fabricated silver nanoparticle against *E. coli* and *S. aureus* (A - 25 µl of AgNPs, B - 50 µl of AgNPs, C - 75 µl of AgNPs, D - 100µl of AgNPs, S – Streptomycin)

Conclusion

The green synthesis of silver nanoparticles using *Illicium Verum* seed extract is an eco-friendly, simple, and efficient route for the synthesis of nanoparticles. They are inexpensive and can produce silver nanoparticles at room temperature. The silver nanoparticles were characterized by UV-visible spectrophotometer, FT-IR, DLS, and SEM analysis. The surface Plasmon resonance of green synthetic silver nanoparticles was confirmed by UV-visible spectral studies. The UV absorption peak at 437 nm clearly indicates the synthesis of silver nanoparticles. The SEM images confirmed that the synthesized nanoparticles were spherical in shape and that their sizes ranged from 100 to 140 nm. It was helpful to analyze their morphology and distribution. FT-IR measurements confirmed that the biomolecules were responsible for reducing and capping silver nanoparticles. The calculated average particle size distribution of silver nanoparticles was found to be 90 nm from the DLS data. The AgNPs biosynthesized from *Illicium Verum* seed extract exhibit excellent anti-bacterial activity against *E. coli* and *S. aureus*. Hence, green synthesis of silver nanoparticles is an effective and eco-friendly process for nanodrugs for pharmacological application as well as large-scale commercial production.

References

1. Aayasha, N., Prachi, S., & Mahender, N. D. S. (n.d.). International journal of basic and applied research A review on eco-friendly synthesis and application of metal nano particles using medicinal plants. Retrieved February 23, 2021, from www.pragatipublication.com
2. Alsalhi, M. S., Devanesan, S., Alfuraydi, A. A., Vishnubalaji, R., Munusamy, M. A., Murugan, K., Nicoletti, M., & Benelli, G. (2016). Green synthesis of silver nanoparticles using Pimpinella anisum seeds: Antimicrobial activity and cytotoxicity on human neonatal skin stromal cells and colon cancer cells. International Journal of Nanomedicine, 11, 4439–4449. https://doi.org/10.2147/IJN.S113193
3. Damayanti, R., Tamrin, Alfian, Z., & Eddyanto. (2020). Preparation of silver nanoparticles from extract of star anise (*Illicium Verum*. Hook. F). AIP Conference Proceedings, 2267, 20065. https://doi.org/10.1063/5.0017822
4. Elamawi, R. M., Al-Harbi, R. E., & Hendi, A. A. (2018). Biosynthesis and characterization of silver nanoparticles using Trichoderma longibrachiatum and their effect on phytopathogenic fungi. Egyptian Journal of Biological Pest Control, 28(1), 28. https://doi.org/10.1186/s41938-018-0028-1
5. Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. In Molecular Aspects of Medicine (Vol. 27, Issue 1, pp. 1–93). Pergamon. https://doi.org/10.1016/j.mam.2005.07.008
6. Heera, P., & Shanmugam, S. (2015). Review Article Nanoparticle Characterization and Application: An Overview. In Int.J.Curr.Microbiol.App.Sci (Vol. 4, Issue 8). http://www.ijcmas.com
7. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. In Journal of HerbMed Pharmacology (Vol. 7, Issue 1, pp. 1–7). Nickan Research Institute. https://doi.org/10.15171/jhp.2018.01
8. Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. In Arabian Journal of Chemistry (Vol. 12, Issue 7, pp. 908–931). Elsevier B.V. https://doi.org/10.1016/j.arabjc.2017.05.011
9. Luna, C., Chávez, V. H. G., Barriga-Castro, E. D., Núñez, N. O., & Mendoza- Reséndez, R. (2015). Biosynthesis of silver fine particles and particles decorated with nanoparticles using the extract of *Illicium Verum* (star anise) seeds. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 141, 43–50. https://doi.org/10.1016/j.saa.2014.12.076
10. Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., Zhang, S. F., Tang, M. K., Sun, J. N., & Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources. In Evidence-based Complementary and Alternative Medicine (Vol. 2014). Oxford University Press. https://doi.org/10.1155/2014/525340
11. Patra, J. K., & Baek, K. H. (2014). Green Nanobiotechnology: Factors Affecting Synthesis and Characterization Techniques. In Journal of Nanomaterials (Vol. 2014). Hindawi Limited. https://doi.org/10.1155/2014/417305
12. Prabhu, K., Sudharsan, P., Kumar, P. G., Chitra, B., & Janani, C. (2022). Impact of Piper betle L. bioactive compounds in larvicidal activity against Culex quinquefasciatus. Journal of Natural Pesticide Research, 2, 100013.
13. Salem, M. A., El-Shiekh, R. A., Hashem, R. A., & Hassan, M. (2021). In vivo Antibacterial Activity of Star Anise (*Illicium Verum* Hook.) Extract Using Murine MRSA Skin Infection Model in Relation to Its Metabolite Profile. Infection and Drug Resistance, Volume 14, 33–48. https://doi.org/10.2147/IDR.S285940

14. Sri, P., Leelavathi, V., Sree, N., & Kumar, M. A. (2015). Antihelmenthic And Antimicrobial Activity Of Green Synthesized Silver Nanoparticles From *Illicium Verum* Hook . F . Fruit.
15. Sudharsan, P., Siva, D., Prabhu, K., & Janani, C. (2022). The Influence of Portulaca oleracea L. Leaves Extracts on the Histoarchitecture of Culex quinquefasciatus and Anopheles stephensi Larvae. Clinical Complementary Medicine and Pharmacology, 100053.
16. Tiquia-Arashiro, S., Rodrigues, D. F., Tiquia-Arashiro, S., & Rodrigues, D. (2016). Application of Nanoparticles. In Extremophiles: Applications in Nanotechnology (pp. 163–193). Springer International Publishing. https://doi.org/10.1007/978-3-319-45215-9_5