Exploration of Green Synthesis of Silver Nanoparticles from Sargassum Wightii and Toxicity Studies in Freshwater Ornamental Fish Poecilia Reticulata

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**Abstract:** This study explores the green synthesis of silver nanoparticles using *Sargassum wightii* seaweed and assesses their toxicity in the freshwater ornamental fish, *Poecilia reticulata*. A visible color shift from yellow to brown confirmed the biosynthesis of AgNPs, while UV-Vis spectroscopy characterization showed a sharp peak at 420 nm, indicating surface plasmon resonance. SEM analysis revealed spherical nanoparticles with sizes below 50 nm, exhibiting clusters, and rough surface textures. EDS confirmed the presence of elemental Ag along with carbon, oxygen, sodium, and chloride in the nanoparticles. Toxicity studies on *P. reticulata* larvae exposed to *S. wightii* extract demonstrated concentration-dependent effects. At lower concentrations (50μg), survival rates were high (98%) throughout 72 hours. However, higher concentrations (75μg) showed reduced survival rates over time, suggesting potential toxicity of *S. wightii* extract on *P. reticulata* larvae. These findings underscore the importance of assessing environmental impacts of AgNP synthesis and their biological effects.

**Keywords:** Cytotoxicity; Green synthesis; Guppy fish; *Poecilia reticulata*; Silver nanoparticles; Survival rate; environmentally friendly

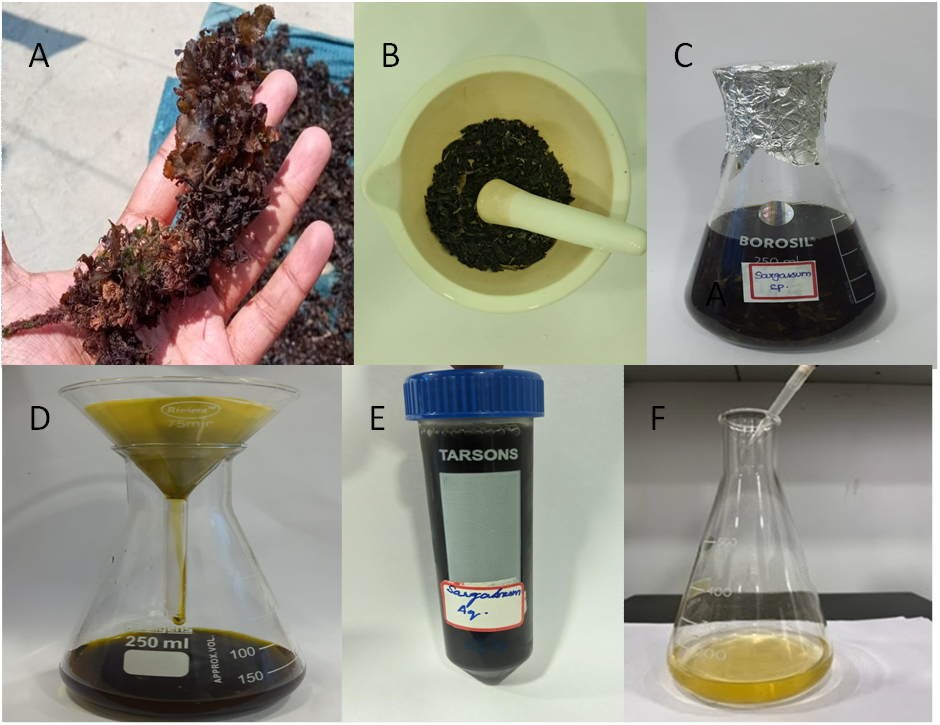
# Introduction

Nanotechnology, which involves the manipulation and application of materials at the nanoscale, has transformed diverse fields such as medicine, electronics, and environmental science. A key breakthrough in this area is the synthesis of nanoparticles, which possess unique chemical, physical, and biological properties due to their high surface area-to-volume ratio and quantum effects[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/EcoXs6/f6nbw+T4TlG+UdjpV). Among various nanoparticles, silver nanoparticles (AgNPs) have attracted significant attention due to their promising bioassay applications (Sharma et al., 2023). The integration of AgNPs with natural resources offers a sustainable approach to enhance their functionality and is viewed as an environmentally friendly and economically advantageous strategy that advances above the other Physico-chemical approaches [(Chaudhari et al., 2023)](https://paperpile.com/c/EcoXs6/suuc). Marine macroalgae, commonly referred to as seaweed, represent a diverse group of photosynthetic organisms that thrive in marine and estuarine ecosystems. They are rich in bioactive compounds, including polysaccharides, proteins, polyphenols, and vitamins, which have significant medicinal, industrial, and nutraceutical applications [(Choudhary et al., 2021)](https://paperpile.com/c/EcoXs6/17qY2). Owing to these bioactive compounds, seaweeds function as dual-purpose reducing and stabilizing agents in the eco-friendly synthesis of nanoparticles [(Lomartire & Gonçalves, 2022)](https://paperpile.com/c/EcoXs6/0e5Zd). *Sargassum* (Phaeophyceae), a globally distributed brown algal genus, is ecologically and economically vital. In India, it is the most diverse genus in Phaeophyta (38 species), with *Sargassum wightii* standing out for its bioactive compound production[*(Helal et al., 2024)*](https://paperpile.com/c/EcoXs6/YvLB6)**.** It is abundant in alginates, fucoidans, and various phenolic compounds, which effectively reduce silver ions to silver nanoparticles [(Shanmugam et al., 2024)](https://paperpile.com/c/EcoXs6/IECC6). Abundant natural polysaccharides, proteins, and polyphenols in seaweed effectively act as stabilizing and reducing agents in this synthesis process [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/EcoXs6/Jv3pq+stU8r+1I3Nn). This eco-friendly approach highlights the potential of seaweed in nanotechnology applications [(Pranati et al., 2021; Sakthi et al., 2021)](https://paperpile.com/c/EcoXs6/zRBG8+uP50K). Freshwater ornamental fish, such as *Poecilia reticulata* (Guppies), are ideal model organisms for toxicity studies due to their sensitivity to environmental changes and widespread use in ecotoxicological research [(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/EcoXs6/pR8Xb+Rh1BZ). Guppy fish is a type of ornamental fish belonging to Cyprinodontiformes(Pratama et al., 2018). The seaweed-based synthesis of AgNPs has gained significant interest recently as a sustainable method. This study enhances the biologically synthesized AgNPs using marine seaweed to analyze the toxic effect on freshwater ornamental fish.

# Materials and Methods

*Sargassum wightii* brown seaweed was collected from the coastline environment of the Gulf of Mannar and transported to the laboratory. (Figure 1a) This freshly collected seaweed was thoroughly rinsed with running water to wash the debris. Subsequently, the cleaned Sargassum wightii was dried in shade and to remove the extra moisture content.

To obtain the aqueous extract, the dried Sargassum wightii was coarsely milled using a mixed grinder, and stored for further analysis(Saadh et al., 2024). (Figure 1b,1c) 20g of powdered Sargassum wightii was added with 100ml of D.H2O in a conical flask and placed in an orbital shaker for 2 days. (Figure 1d) Afterward, the extract underwent filtration using Whatman filter paper and stored for further analysis according to [(Abdi et al., 2018)](https://paperpile.com/c/EcoXs6/rn0Ms). AgNPs were synthesized by combining 20 mL of S. wightii aqueous extract with 90 mL of 20 mM AgNO₃. The mixture was stirred continuously at room temperature for 90 min. Nanoparticle formation was preliminarily indicated by a color shift (Figures 1e,f). Biosynthesized Silver nanoparticles were transparent and the color changed from yellow to brown, a clear indication that the AgNPs were formed and given to characterization analysis (Almatrafi et al., 2024). The synthesized sample was centrifuged at 5000 rpm at 15 mins. The AgNP pellets formed after centrifugation and discard the Supernatant, were oven-dried at 50ºC [(Abdi et al., 2018)](https://paperpile.com/c/EcoXs6/rn0Ms). We then milled the sample using mortar and pestle and stored for further Characterization.

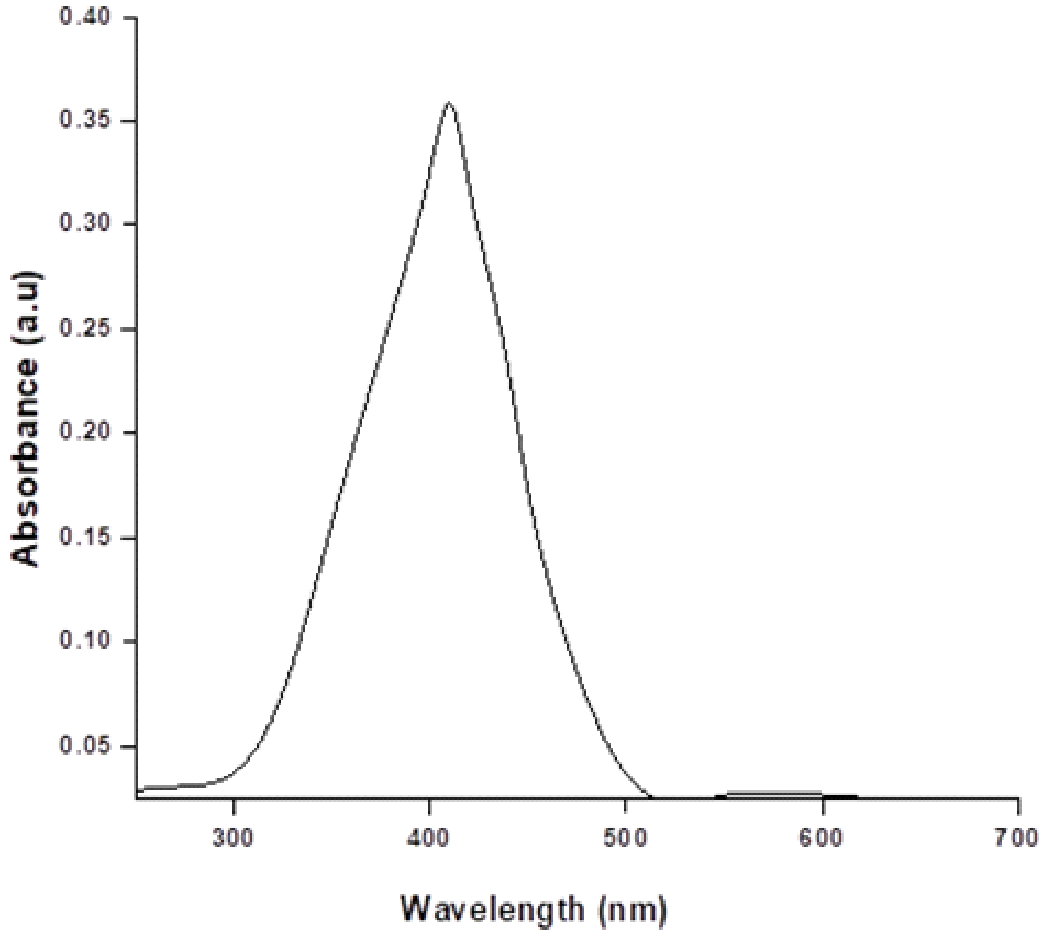


**Figure 1.** Biosynthesis of AgNPs: (A) *S. wightii* Seaweed (B) *S. wightii* Seaweed Powdered sample (C) and (D) Extraction and Filtration of seagrass (E) Aqueous extract of Seaweed sample (F) Synthesis of Silver nanoparticle

UV-visible spectroscopy confirmed AgNP biosynthesis via the characteristic plasmon resonance peak, correlating with the observed color change. FTIR analysis revealed functional groups critical for reduction and capping, while SEM imaging delineated particle morphology and size distribution. EDX spectroscopy further authenticated the elemental signature of the nanoparticles [(Tharani et al., 2023)](https://paperpile.com/c/EcoXs6/Y9KGV). *oecilia reticulata* were purchased from kolathur, market and transported to acclimatize for 15 days at the Marine Biomedical Research Lab and Environmental Toxicological Unit. Acute toxicity tests were performed with the *Poecilia reticulata* (Guppy fish) was followed in the 96-hour acute toxicity test of guppy fish larvae on *Sargassum wightii* crude extract. For every treatment, larvae of guppy fish in the same age group and size were chosen. The crude extract concentrations of 50, 75, 100 μg/ml were added to the experimental tanks, and the temperature was kept at 25°C for the duration of the experiments. Ten fish per treatment were used in total for the five treatments. The control group which is untreated and the seaweed crude extract at the previously specified concentrations were observed. Mortality was assessed by recording the cumulative number of fish that died in each treatment group after 96 hours.

# Results

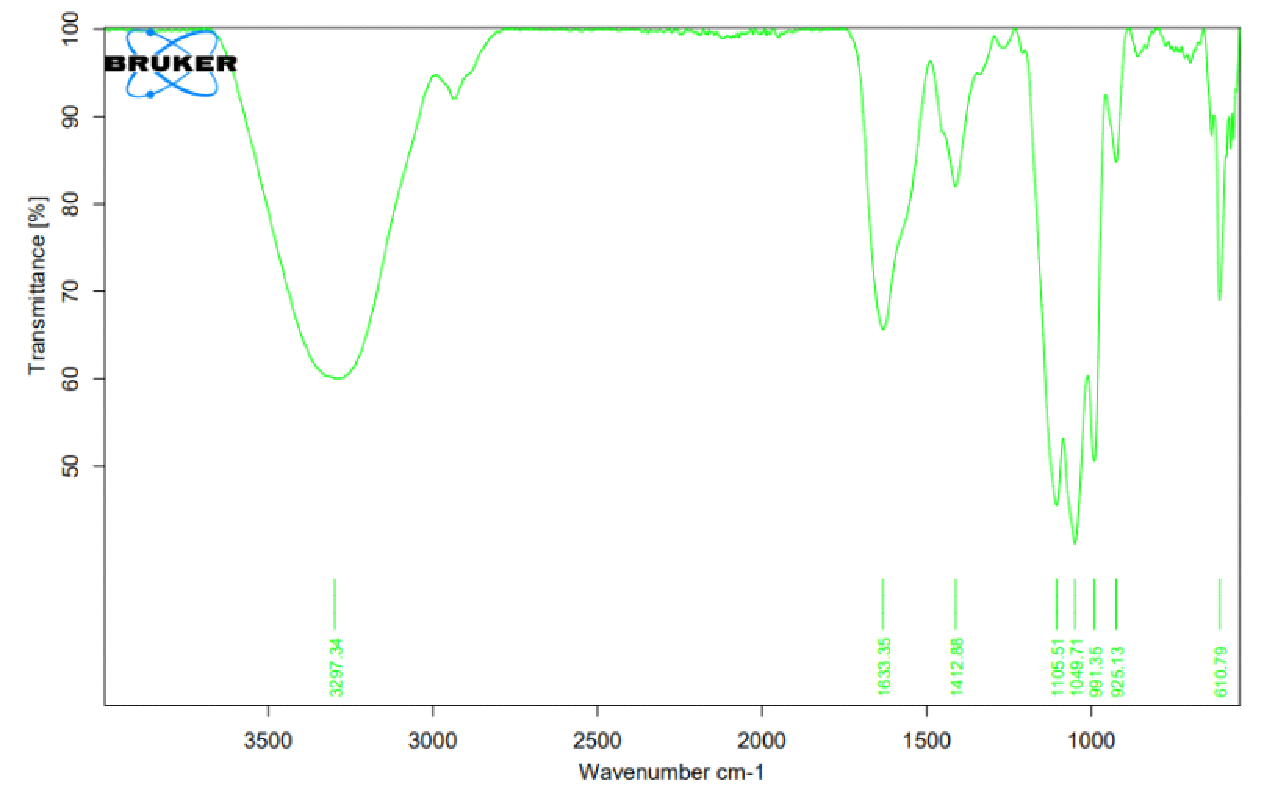
The aqueous extract of brown seaweed facilitated the eco-friendly biosynthesis of silver nanoparticles (AgNPs) through the reduction of Ag⁺ ions, as evidenced by a rapid color change in the reaction mixture. UV-Visible spectroscopic analysis confirmed the formation of AgNPs, revealing a sharp surface plasmon resonance band centered at 420 nm (Figure 2), which is characteristic of spherical AgNPs. The high intensity and symmetry of the absorbance peak suggest a monodisperse size distribution with minimal aggregation. The narrow full-width-at-half-maximum (FWHM) of the SPR band further corroborates the uniformity in nanoparticle morphology and colloidal stability. These spectral features align with established reports of biologically synthesized AgNPs, underscoring the efficacy of seaweed-derived reductants in nanoparticle formation.



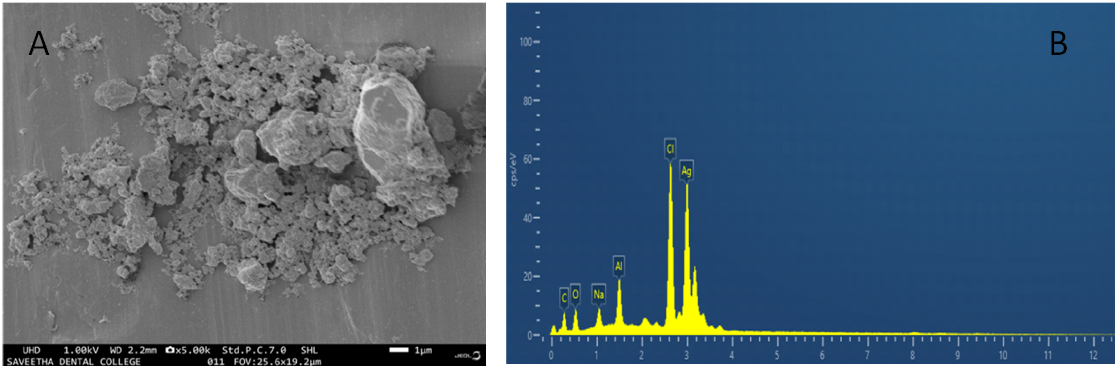
**Figure 2.** UV-visible spectrophotometer of synthesized Silver nanoparticle

The FTIR spectrum of AgNPs synthesized from *Sargassum wightii* revealed several characteristic absorption bands indicative of various functional groups (Figure 3). Strong and sharp peak at 3297.34 cm⁻¹ is attributed to C-H stretching in alkynes, suggesting the presence of triple-bonded hydrocarbons. The medium intensity peak at 1633.35 cm⁻¹ corresponds to C=C stretching in alkenes, indicating the presence of double-bonded carbon structures. A prominent peak at 1412.88 cm⁻¹ is assigned to S=O stretching in sulfates, reflecting the incorporation of sulfate groups. The strong band at 1105.51 cm⁻¹ is due to C-O stretching in aliphatic ethers, revealing the presence of ether linkages. Additionally, a strong, broad absorption at 1049.71 cm⁻¹ corresponds to CO-O-CO stretching in anhydrides, indicating the presence of anhydride groups. The spectrum also displayed a strong band at 991.35 cm⁻¹, which is attributed to C=C bending in alkenes, suggesting the presence of bending vibrations of double-bonded carbons. Finally, the strong absorption band at 610.79 cm⁻¹ corresponds to C-Br stretching, indicating the presence of halo compounds. This identification provides crucial evidence of the organic components contributing to the formation of AgNPs and supports the utilization for nanoparticle synthesis.

SEM analysis, a surface imaging method, was utilized to evaluate the morphology and size of the synthesized nanoparticles. The surface texture of the nanoparticles is rough, which may influence their surface area and reactivity. The distribution is non-uniform, with densely packed regions and areas with fewer particles. (Figure 4a) The result of the SEM image showed the morphological characterization of biosynthesized seaweed *Sargassum* wightii. SEM analysis distributed silver nanoparticles spherically with clusters together that had size below 50 nm. The synthesized silver nanoparticles (AgNPs) displayed well-defined cubic morphology, with an average particle size distribution of 37–60 nm, as evidenced by high-resolution imaging (Figure 4b). Elemental composition analysis via energy-dispersive spectroscopy (EDS) revealed strong signals corresponding to metallic silver (Ag), with characteristic Lα and Lβ emission peaks at 2.98 keV and 3.15 keV, respectively. These dominant peaks confirm the successful reduction of Ag⁺ to elemental silver (Ag⁰) during synthesis. Minor spectral signatures of carbon (C), oxygen (O), sodium (Na), and chloride (Cl) were also detected, likely originating from residual biomolecules in the seaweed extract that contributed to nanoparticle stabilization. The absence of impurity-related peaks underscores the high purity of the synthesized AgNPs. The intensity and sharpness of these peaks suggest that silver is the dominant component in the nanoparticles. This absorption range corresponds to the optical characteristics of surface plasmon resonance.

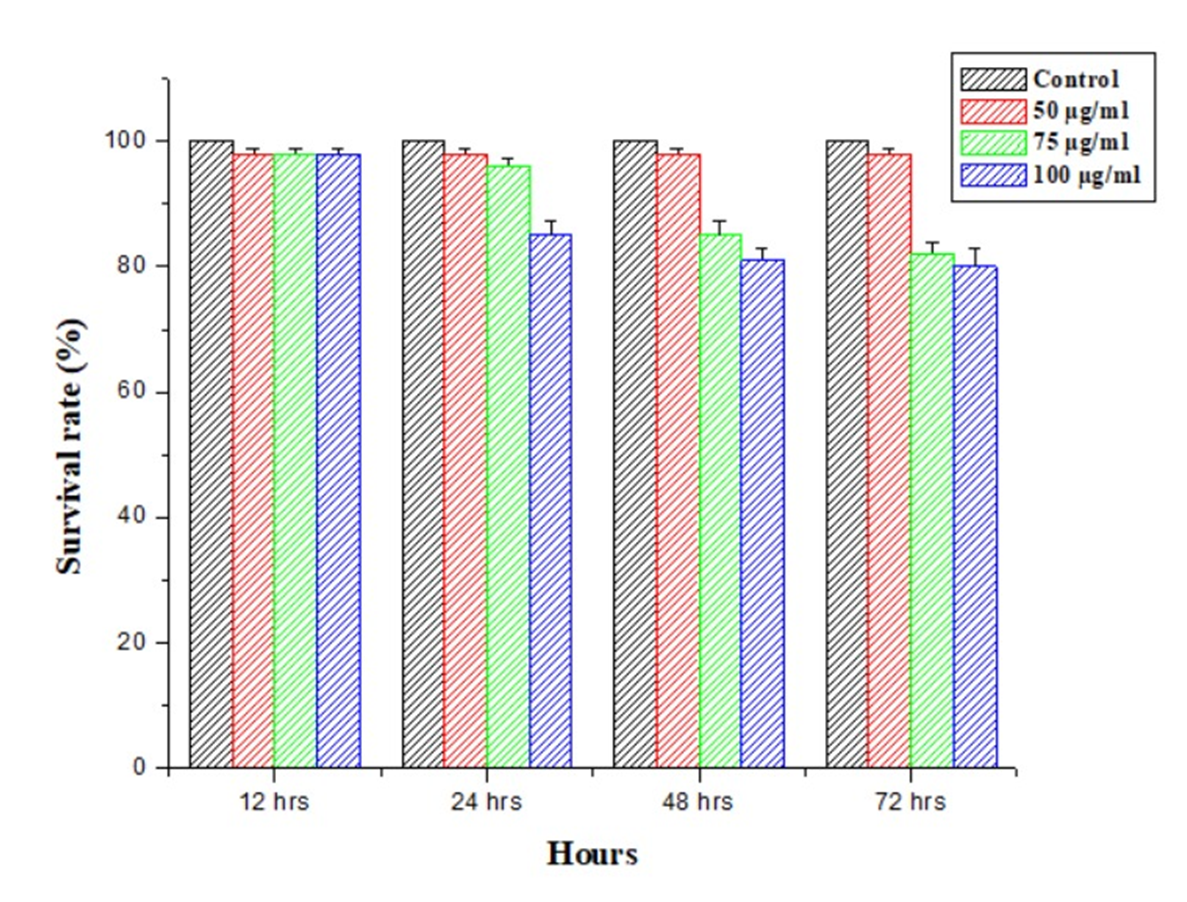


**Figure 3.** FTIR analysis of synthesized Silver nanoparticles



**Figure 4.** (A) and (B) SEM and EDX image of synthesized Silver nanoparticles

Toxicity studies were conducted on Guppy fish larvae (*Poecilia reticulata*) using crude extract of *Sargassum wightii*. The survival rates of the larvae were monitored at different time intervals 12, 24, 48, and 72 hours across different concentrations of the extract (Figure 5). The control group, which was not exposed to the extract, exhibited 100% survival throughout the study period, indicating no adverse effects from handling or environmental conditions. At 50μg concentration of *Sargassum wightii* extract, the survival rate remained, increasing the concentration to 50μg and maintaining a similar survival rate of 98% with a standard deviation of ±0.8 at all-time intervals. However, higher concentrations showed a dose-dependent decrease in survival rates at 75μg concentration, the survival rates decreased slightly to 98% at 12 hours but decreased to 82% at 72 hours, with standard deviations ranging from ±0.8 to ±2.2. Further increasing the concentration to 100μg resulted in a more pronounced decrease in survival rates from 85% at 24 hours to 80% at 72 hours, with standard deviations ranging from ±1.9 to ±3. These results suggest that *Sargassum wightii* crude extract will induce toxicity in *Poecilia reticulata* larvae in a concentration-dependent manner, with higher concentrations leading to decreased survival rates over time. The toxicity assessment of the synthesized AgNPs was conducted on the freshwater ornamental fish *Poecilia reticulata*. The concentration-dependent toxicity observed in *Poecilia reticulata* suggests that while AgNPs have promising applications, their environmental impact should be carefully evaluated, especially when released into aquatic systems.



**Figure 5.** Survival rate of Freshwater Ornamental Fish *Poecilia reticulata*

# Discussion

The yellow-to-brown color shift and 420 nm SPR peak confirm AgNP formation, attributable to the collective oscillation of conduction electrons in spherical nanoparticles. This aligns with SPR ranges reported for other seaweed-synthesized AgNPs (409–429 nm), suggesting that algal bioactive compounds universally facilitate Ag⁺ reduction despite taxonomic differences in source species [(Thiurunavukkarau et al., 2022)](https://paperpile.com/c/EcoXs6/voLEQ); [(Bhuyar et al., 2020)](https://paperpile.com/c/EcoXs6/s2AY8). Similarly, AgNPs synthesized from *C. myrica*, *G. foliifera* and *U. rigida,* displayed absorption peaks at 409 nm, 415 nm, and 424 nm, respectively. These consistent peaks indicate that the nanoparticles are spherical and well-dispersed, without significant aggregation, as supported by the sharpness and symmetry of the UV-Vis absorption spectra [(Agustina et al., 2021)](https://paperpile.com/c/EcoXs6/NJzBB). Fourier Transform Infrared spectroscopy further elucidated the presence of organic compounds capping the AgNPs[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/EcoXs6/81seW+p0kkr+q9GMr)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/EcoXs6/81seW+p0kkr+q9GMr+UTdEd). The sharp peak at 3297.34 cm⁻¹, attributed to C-H stretching in alkynes, suggests the incorporation of triple-bonded hydrocarbons, likely derived from fatty acids or alkyne derivatives [(Chugh et al., 2021)](https://paperpile.com/c/EcoXs6/ok8aC). Additionally, the medium intensity band observed at 1633.35 cm⁻¹, corresponding to C=C stretching in alkenes, points to the presence of double-bonded carbon structures commonly found in unsaturated fatty acids and aromatic compounds [(Bamal et al., 2021)](https://paperpile.com/c/EcoXs6/ssclH). A prominent peak at 1412.88 cm⁻¹, associated with S=O stretching in sulfates, indicates the involvement of sulfate groups, possibly from polysaccharides within the seaweed matrix [(Jain et al., 2021)](https://paperpile.com/c/EcoXs6/VVlgG). These spectral features underscore the role of organic components derived from seaweed extract in the stabilization and formation of AgNPs[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/EcoXs6/j6xIZ+XkyXI+eg6Oj). Scanning electron microscopy (SEM) analysis confirmed the spherical morphology of the biosynthesized AgNPs, with a particle size distribution predominantly below 100 nm. This observation is consistent with previous studies, such as those conducted on Sargassum polycystum and Colpomenia sinuosa, where the nanoparticles were found to be within the size range of 54 to 85 nm [(Vishnu Kiran & Murugesan, 2020)](https://paperpile.com/c/EcoXs6/X9a0K); [(Thiurunavukkarau et al., 2022)](https://paperpile.com/c/EcoXs6/voLEQ). Furthermore, EDS confirmed the presence of elemental silver, with significant peaks at approximately 2.98 keV and 3.15 keV, corresponding to the Lα and Lβ lines of silver, respectively [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/EcoXs6/vPdJn+CEvmb+u8NUS). The presence of characteristic silver signals, along with the detection of associated elements (carbon, oxygen, sodium, and chloride), confirms the successful biosynthesis of silver nanoparticles [(Francis et al., 2018)](https://paperpile.com/c/EcoXs6/Xli10). Previous study demonstrated that the Sargassum wightii crude extract-induced AgNPs exhibited a concentration-dependent toxicity in P. reticulata larvae, with higher concentrations leading to decreased survival rates over time. This finding is in line with the work of [(Mohsenpour et al., 2020)](https://paperpile.com/c/EcoXs6/JIKBc), who observed a significant decline in the survival rate of guppies as the concentration of AgNPs increased. The study further revealed that the mortality rate in Poecilia sphenops and their larvae had a positive correlation with AgNP concentration, with LC50 96 h values of 6.22 mg L−1 for larvae and 26.85 mg L−1 for adult, highlighting the higher sensitivity in larvae [(Vali et al., 2022)](https://paperpile.com/c/EcoXs6/23vHU). These findings highlight significant ecotoxicological concerns regarding silver nanoparticle applications, especially in sensitive aquatic ecosystems.

# CONCLUSION

In Conclusion, the present study demonstrates that nanotechnology has driven the synthesis of AgNPs, important in medicine, electronics, and environmental science. Seaweeds like *Sargassum wightii*, rich in polysaccharides and polyphenols, efficiently biosynthesized AgNPs, offering a sustainable alternative. Toxicity studies on *P. reticulata* larvae exposed to *S. wightii* extract demonstrated concentration-dependent effects on survival rates, underscoring potential ecological risks associated with AgNP exposure in aquatic environments. This comprehensive approach underscores the importance of assessing both the synthesis and environmental impact of AgNPs, providing insights for future research on their safe and sustainable applications.

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