Biosynthesis of Gold Nanoparticles Using Seagrass Halophila decipiens and Larval Toxicity Assessment in Siamese Fighting Fish (Betta splendens)

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**Abstract:**The biosynthesis of gold nanoparticles (AuNPs) with aqueous extract of *Halophila decipiens* was successfully achieved, evidenced by colour change from yellow to red and a UV-vis spectral peak at 523 nm. EDX confirms that significant gold peaks at higher energy levels are present, while Fourier-transform infrared spectroscopy (FTIR) identifies various functional groups, including alcohols, ethers, and aromatic compounds. Scanning electron microscopy (SEM) analysis revealed predominantly spherical nanoparticles with diameters ranging from approximately 10 to 50 nm. Toxicity studies indicated that lower concentrations of AuNPs (25 and 50 µg/ml) had minimal impact on larval survival, whereas higher concentrations (75 and 100 µg/ml) significantly reduced survival rates and increased variability. These findings suggest that larvae experience substantial physiological stress and heightened susceptibility to the toxic effects of AuNPs with prolonged exposure. Future research focuses on further exploring the safety profile, optimizing synthesis methods, and advancing towards clinical trials to harness the therapeutic benefits of marine-derived nanoparticles.

**Keywords:**Gold nanoparticles, *Halophila decipiens,* FTIR, SEM, *Betta splendens*, larvae toxicity

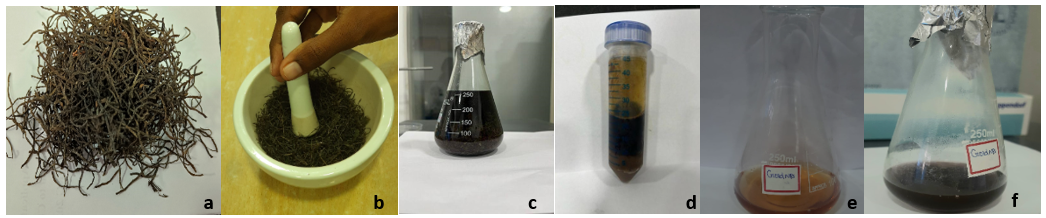
# Introduction

Nanotechnology holds immense promise for innovation, addressing nanotoxicity is crucial for maximizing benefits and minimizing risks to human health and the environment ([(Ali et al., 2021)](https://paperpile.com/c/mNsN7N/TYJ7I). Gold nanoparticles occupy a prominent position among nanoparticles due to their exceptional optical, electronic, and catalytic characteristics, making their synthesis a cornerstone in nanotechnology, employing various chemical, physical, and biological methods to precisely control size, shape, and surface properties [(Sibuyi et al., 2021)](https://paperpile.com/c/mNsN7N/n3Ruj). Gold nanoparticles (AuNPs) exhibit promising candidates for diverse biological applications, especially advantageous due to their synthesis methods that minimize reliance on toxic chemicals for reduction purposes [(Patil et al., 2023)](https://paperpile.com/c/mNsN7N/RhzBS). The widespread adoption of eco-friendly procedures for green synthesis has opened up promising novel avenues for a variety of biological applications involving Gold (Au) nanoparticles (NPs) [(Ahmad et al., 2024)](https://paperpile.com/c/mNsN7N/zsxGV). The biosynthesis of gold nanoparticles (AuNPs) mediated by seagrass involves using extracts or components from seagrass species as reducing and stabilizing agents in the synthesis process [(Chaudhary et al., 2020)](https://paperpile.com/c/mNsN7N/lQFYD). Some compounds extracted from seagrasses, including *Halophila decipiens*, have shown antimicrobial activity against various pathogens, suggesting potential applications in medicine and biotechnology [(Utami et al., 2024)](https://paperpile.com/c/mNsN7N/6NTaN). Sublethal concentrations, accumulated by fish through dietary intake or direct exposure, can induce potential toxic effects over time and alter their behaviour, including feeding habits, reproductive behavior, and interactions within predator-prey dynamics [(Devasena et al., 2022)](https://paperpile.com/c/mNsN7N/ARxEW). *Betta splendens*, commonly known as the Siamese fighting fish, has been extensively bred for both its aggressive behavior in males, selected for fighting ability, and for ornamental traits such as vibrant coloration, distinctive fin shapes, and body size with unique characteristics, coupled with its evolutionary lineage, position [(Srikulnath et al., 2021)](https://paperpile.com/c/mNsN7N/mvboc). *Betta splendens* as a valuable model organism for studying a wide range of research fields including behavior, endocrinology, neurobiology, genetics, developmental biology, and evolutionary biology [(Lichak et al., 2022)](https://paperpile.com/c/mNsN7N/7VE1f). The potential toxicity of AuNPs to larvae raises concerns about their impact on marine ecosystems, potentially affecting fish species dependent on seagrass habitats through reduced larval survival and impaired development, thereby disrupting trophic interactions and food web dynamics within coastal ecosystems [(E. M. N. Oliveira et al., 2020)](https://paperpile.com/c/mNsN7N/RMiGW). This research underscores studies on seagrass-mediated gold nanoparticles and larvae toxicity highlight the intricate interaction between nanoparticles, seagrass habitats, and marine organisms.

# Materials and Methods

Samples were collected from Rameshwaram and sent to the Marine Biomedical Laboratory and Environmental Toxicology Unit at the It is rinsed with freshwater to remove impurities. Thereafter, the seagrass underwent additional cleaning followed by two washes with distilled water. After thorough cleaning, it is air dried at 37°C for 5-6 days. Dried samples were then grinded into powder form and stored.

10 grams of *Halophila decipiens* powder was mixed in 400 ml of d.H2O and heated at 70°C for 15 min. Resulting mixture filtered through Whatman paper to remove precipitates, with the filtration process repeated three times until clear extracts were obtained. These extracts were stored at 4°C for further process.10 grams of dried *Halophila decipiens* powder were immersed in 200 ml of 70% ethanol along with agitation at room temp. for 24 hours. The mixture was then filtered using Whatman filter paper no 1, and the filtrate was concentrated, dried using a rotary evaporator set at 45°C.To synthesize *Halophila decipiens* mediated gold nanoparticles (AuNPs), 100 mL of aqueous *Halophila decipiens* extract mixed with 40 mL of AuCl4 solution. The mixture was mixed properly at 37°C using a magnetic stirrer. This was indicated by a color change, signifying reduction of gold ions, which was periodically monitored using a UV-Vis spectrophotometer. After confirming the presence of Au NPs, the solution then centrifuged for 30 mins at 5000 rpm. The resulting pellet which contains the nanoparticles then collected, dried in a hot air oven for 8 hours at 90°C to obtain the final sample. Overall Summary of AuNPs synthesis is shown in figure 1.



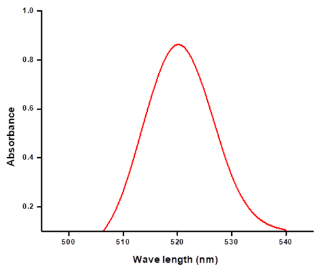
**Figure 1.** Summary of Synthesis of Gold Nanoparticles: (a) *Halophila decipiens* Seagrass (b) *Halophila decipiens* Seagrass Powdered sample (c) Extraction of seagrass (d) Aqueous extract of seagrass (e) Initial stage of AuNPs synthesis (f) Final stage of AuNPs synthesis

Characterizing synthesized gold nanoparticles involves assessing physicochemical features such as size, size distribution, surface morphology, specific surface area, agglomeration, shape, aggregation state, as well as structural characteristics including crystallinity and defect structure. UV-Vis spectroscopy is commonly used to determine the surface plasmon resonance of gold nanoparticles (AuNPs), which appears as a characteristic absorption peak around 520 nm due to collective oscillation of free electrons in the nanoparticles [(Ngumbi et al., 2019)](https://paperpile.com/c/mNsN7N/TwPAp). FTIR can be used to monitor chemical reactions involving AuNPs. Changes in FTIR spectra before and after a reaction can indicate chemical transformations or modifications of the nanoparticles [(Sakellari et al., 2020)](https://paperpile.com/c/mNsN7N/HPONi). (SEM) scanning electron microscopy is an effective technique for characterizing synthesized gold nanoparticles (AuNPs), providing detailed information on their morphology, size distribution, and aggregation state [(Clarance et al., 2020)](https://paperpile.com/c/mNsN7N/ansN2). EDX technique offers qualitative data, semi-qualitative data, and quantitative data, along with area distribution through element mapping of synthesized gold NPs and also estimates the elemental distribution within the solution [(Mat Isa et al., 2022)](https://paperpile.com/c/mNsN7N/RHI7W).

*Betta splendens* larvae were cultured under standardized conditions, maintaining controlled temperature and humidity to ensure uniformity in size and health. Stock solutions of the substance were prepared at different concentrations of (50, 75 and 100 μg/ml) using distilled water as a solvent (Almatrafi et al., 2024). Control group was established using only the solvent to account for non-substance-related effects(Saadh et al., 2024). Ten larvae per concentration were transferred into separate Petri dishes containing both the test solutions and the control solution, with replicates for statistical analysis. Petri dishes were then incubated under suitable environmental conditions, including temperature for the larvae species. Observations were conducted every 12 hours over 72 hours to record mortality rates, and morphological changes. Data analysis involved calculating larval mortality percentages and assessing developmental abnormalities for each concentration and the control group

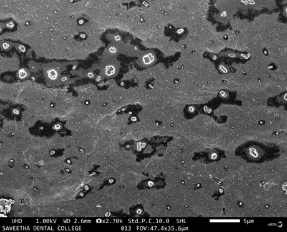
# Results

Characterizing the synthesized gold nanoparticles is essential for understanding their size, shape, crystallinity, and surface properties. A variety of analytical techniques, such as UV-visible spectrophotometry, Energy Dispersive X-ray spectroscopy, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared spectroscopy-FTIR will be employed to examine the physicochemical characteristics of the nanoparticle.The UV-Vis spectroscopic analysis shows a distinct absorbance peak centred at approximately 523 nm presented in figure 2. This peak is indicative of the surface plasmon resonance (SPR) of gold nanoparticles (AuNPs). The high absorbance at this wavelength confirms the successful synthesis of AuNPs, which is characterized by colour change from yellow to red due to excitation of surface plasmon vibrations.



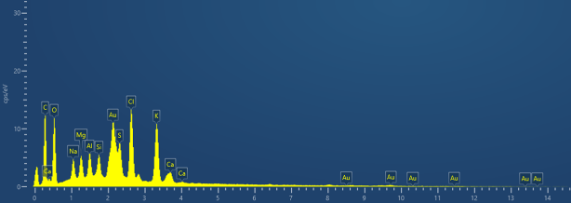
**Figure 2.** UV- visible spectrophotometer of synthesized gold nanoparticles shows distinct absorbance peaks centered at approximately 523 nm

The gold nanoparticles are predominantly spherical, with diameters ranging from approximately 10 to 50 nm as shown in figure 3. Some particles exhibit slight deviations from spherical shapes, which might be due to aggregation or non-uniform growth during the synthesis process. The SEM results reveal a uniform distribution of AuNPs, although the surface shows some degree of aggregation, resulting in random aggregates of varying sizes and irregular shapes.



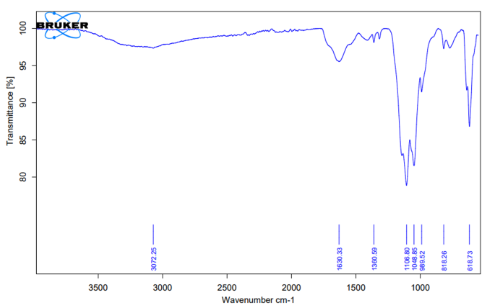
**Figure 3.** SEM image of synthesized gold nanoparticles particles exhibit slight deviations from spherical shapes with diameters ranging from approximately 10 to 50 nm

The EDX spectrum indicates that the sample contains gold nanoparticles, evidenced by the multiple Au peaks at higher energy levels mentioned in figure 4. These peaks are characteristic of the gold element and confirm its presence in significant quantities. Presence of other elements such as C, Mg, Na, Al, S, Si, Cl, Ca, K, and O suggests that the sample may be in a complex matrix or contains impurities from the synthesis process or the substrate on which the gold nanoparticles were deposited. Similar outcomes of intense signal from gold atoms that confirms the successful synthesis of AuNPs using leaf extracts of Cymbopogon citratus, and Pistia stratiotes.



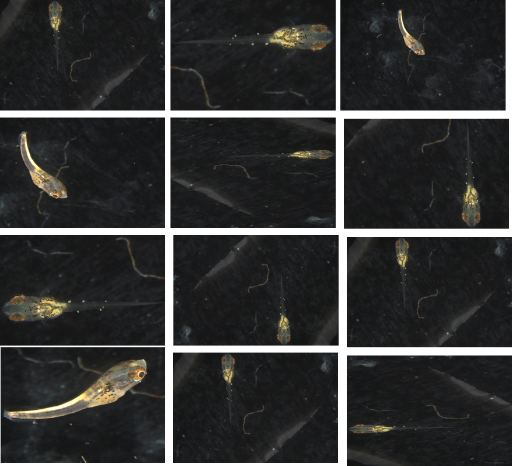
**Figure 4.** EDX image of synthesized gold nanoparticles evidenced by Au peaks at higher energy levels along with Presence of other elements such as C, Mg, Na, Al, S, Si, Cl, Ca, K, and O.

FT-IR spectrum of the gold nanoparticles reveals several characteristic peaks that provide insights into the chemical environment and surface chemistry of the nanoparticles shown in figure 5. The peak at 3027.25 cm⁻¹ could be attributed to C-H stretching frequency, indicating the presence of organic compounds, possibly from capping agents or solvents used in the synthesis process. The peak at 1635.30 cm⁻¹ is typically associated with the bending vibrations of water (H-O-H) or C=O shifting vibrations, suggesting the presence of water or carbonyl groups. Peak at 1509.99 cm⁻¹ indicate aromatic C=C stretching vibrations, suggest that aromatic compounds are present. Also, the peak at 1384.90 cm⁻¹ can be attributed to the bending vibrations of CH₃ groups, indicating the presence of methyl groups. The peak at 1030.89 cm⁻¹ might correspond to C-O stretching vibrations, indicate that alcohols, ether or esters are also present while the nearby peak at 1018.85 cm⁻¹ is also likely related to C-O stretching or C-H bending vibrations. Peaks at 849.92 cm⁻¹ and 818.78 cm⁻¹ are typically associated with bending vibrations of aromatic C-H bonds. Lastly, the peak at 614.73 cm⁻¹ might be related to metal-oxygen (M-O) vibrations, which can be indicative of interactions between the gold nanoparticles and oxygen-containing groups.



**Figure 5.** FTIR analysis of synthesized gold nanoparticles identified various functional groups, such as alcohols, ethers, and aromatic compounds.

Survival rates of Siamese Fighting Fish (*Betta splendens*) are observed at 12 hour intervals (12, 24, 48, and 72 hours) accompanied by standard deviations. In the control group, the fish survival rate remains consistently at 100% across all time points (12, 24, 48, and 72 hours) with a very low standard deviation of 0.1 which indicates that the control conditions are optimal and stable, with no mortality and minimal variability as mentioned in figure 6. Fish survival rate is highly dependent on the concentration of the tested substance. At lower concentrations (50μg), the survival rate remains high with minimal impact. Where, at higher concentrations (100μg), there is dose-dependent impact in survival rates, accompanied by increasing variability. Results suggest that the substance exhibits toxicity at higher concentrations, significantly affecting fish survival and indicating potential risks associated with higher exposure levels.



**Figure 6:**Toxicity study on Siamese larvae indicates that lower concentrations of AuNPs had minimal impact on larval survival and at higher concentrations, the effect remained minimal.

# Discussion

Previous study indicates that the synthesized gold nanoparticles (AuNPs) are characterized by a distinct single Surface Plasmon Resonance (SPR) band at 527 nm in UV-visible spectra [(Sathiyaraj et al., 2021)](https://paperpile.com/c/mNsN7N/CNRvB). UV-Vis spectroscopic analysis, colour change from yellow to red observed during synthesis of AL-AuNPs is attributed to the excitation of surface plasmon vibrations. A prominent absorption peak at 535 nm confirmed the presence of AuNPs [(Soto et al., 2021)](https://paperpile.com/c/mNsN7N/D7lMY).Additionally, high-magnification images indicate the formation of larger particles and increased agglomerations [(A. E. F. Oliveira et al., 2023)](https://paperpile.com/c/mNsN7N/P6wj). The SEM analysis reveals that the gold nanoparticles exhibit a size range of 3-4 µm. Morphologically, the particles predominantly display a spherical shape, though many are irregular[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/mNsN7N/oNEcE+RXxTH+5cT9P). Additionally, the nanoparticles tend to agglomerate, forming clusters. Despite this aggregation, the particles are largely uniform in both shape and size [(Netam et al., 2021)](https://paperpile.com/c/mNsN7N/3cXn0).In the EDX profile, signals like oxygen and carbon likely originated from organic molecules or phenolic compounds present on the nanoparticle surfaces [(Hatipoğlu, 2021)](https://paperpile.com/c/mNsN7N/0CEOX). Previous study to the EDX shows the gold composition by mass is 11.13%[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/mNsN7N/9WTcm+ii5nF+3B6yM). The presence of carbon and oxygen peaks in the spectrum suggests the existence of phytoconstituents and organic capping agents derived from the upland cress extract[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/mNsN7N/3WELc+V2l5k+3mDY1). Additionally, other inorganic elements such as Ca, potassium, chlorine, and Mg were detected, likely originating from their high content in upland cress [(Hutchinson et al., 2021)](https://paperpile.com/c/mNsN7N/VaSQT).FTIR analysis identifies specific biomolecules crucial for synthesizing gold nanoparticles. Vibrational stretches at 1644 cm⁻¹ indicate alkenes (strong), 1399 cm⁻¹ represent CH₃ bends, 3542 cm⁻¹ signify OH stretch, 2926 cm⁻¹ denote C–H stretches, 1066 cm⁻¹ correspond to C-F bonds and 557 cm⁻¹ indicate C-Br bonds. Functional groups are crucial in the reduction or stabilization of gold nanoparticles (AuNPs) synthesized by bacteria, frequently facilitated by enzymes or proteins [(Shunmugam et al., 2021)](https://paperpile.com/c/mNsN7N/4GwyW). Significant peaks were observed at 3367.69 cm⁻¹ and 3359.27 cm⁻¹, indicating presence of CO-OH and O-H groups[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/mNsN7N/e211c+xDP9o+WhEu3). These peaks were attributed to alcohols, aldehydes, amines, metabolites and proteins found in leaves of *C. wightii*. Furtherly, the band at 1636 cm⁻¹ in both spectra corresponds to the amide I linkage, indicative of the carbonyl stretches in proteins [(Uzma et al., 2020)](https://paperpile.com/c/mNsN7N/gWPkz).In contrast of previous study, author reported after 21 days of exposure, prometryn at concentrations of 1, 10, 100, and 1000 μg/L significantly increased the larval death rate to 28.51 ± 2.8%, 43.62 ± 3.9%, 54.24 ± 1.3%, and 67.07 ± 6.1% respectively, prometryn has a detrimental effect on larval survival and development, impacting various morphological parameters in a dose-dependent manner[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/mNsN7N/WA07Q+qiK8i+C4qMD)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/mNsN7N/WA07Q+qiK8i+C4qMD+OBmPl). Changes in body length, width, weight, and specific anatomical features such as eye diameter and fin distance suggest that prometryn disrupts normal growth and development in larvae, leading to significant physiological stress and potential long-term consequences for the population [(Samreen et al., 2022)](https://paperpile.com/c/mNsN7N/JV5eb). Further result shows that when larvae exposed to lead at 48 hrs various abnormalities were observed in exposed groups. These phenotypic abnormalities included spinal and tail deformities, yolk swelling and pericardial edema[(Pranati et al., 2021; Sakthi et al., 2021](https://paperpile.com/c/mNsN7N/hGejm+gS8hQ)). After 96 hours, occurrence of yolk sac swelling and pericardial edema increased significantly compared to those at lower concentrations. By 144 hours of exposure, every individual exhibited spinal and tail deformities [(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/mNsN7N/Pbpk4+bwAEg)). Additionally, larvae frequency of displaying pericardial edema and yolk sac swelling was increased, indicating a dose-dependent escalation in deformities with prolonged exposure to lead [(Curcio et al., 2021)](https://paperpile.com/c/mNsN7N/nTXHJ).

# Conclusion

The study successfully achieved the biosynthesis of Gold Nanoparticles using an aqueous extract of *Halophila decipiens*, evidenced by a distinct color change and a characteristic UV-vis spectral peak. Energy dispersive X-ray spectroscopy confirmed the presence of gold, and Fourier Transform Infrared spectroscopy identified various functional groups, such as alcohols, ethers, and aromatic compounds. Scanning electron microscopy revealed that the nanoparticles were predominantly spherical. Toxicity studies indicated that lower concentrations of AuNPs had minimal impact on larval survival, and even at higher concentrations, the effect remained minimal. This suggests that while larvae experience physiological stress and increased susceptibility to the toxic effects of AuNPs with prolonged exposure, the impact is relatively limited. Thus, biosynthesis of gold nanoparticles using seagrass was successfully achieved, and their larval toxicity assessment in Siamese Fighting Fish (*Betta splendens*) revealed that while the nanoparticles exhibit minimal toxicity at lower concentrations, higher concentrations pose moderate risks, affecting fish survival. This study highlights the potential ecological impact of gold nanoparticles and underscores the need for careful consideration of their environmental exposure levels.

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