Genotoxicity Assessment of Syringodium Isoetifolium Seagrass Extract in Ornamental Siamese Fighting Fish (Betta Splendens)

S Deepika1 , T.Savitha1,a)

1Deepika Herbal Centre, Hyderabad, Telangana, India

**Corresponding Author:** a)[tanishkaasaveetha777@gmail.com](mailto:tanishkaasaveetha777@gmail.com)

**Abstract:** This study explores the phytochemical composition, functional group identification, and genotoxicity assessment of the ethanolic crude extract of Syringodium isoetifolium. Phytochemical analysis confirmed the presence of saponins, tannins, flavonoids, and alkaloids, which are associated with antioxidant and antimicrobial properties, while steroids and terpenoids were absent. FTIR spectroscopy identified characteristic absorption peaks, including O-H stretching at 3305 cm⁻¹ and C=O stretching at 1648 cm⁻¹, indicating the presence of alcohols, phenols, alkanes, ethers, and carbonyl compounds. These findings are consistent with previous research on seagrass species, which also detected bioactive compounds like flavonoids and phenols. Genotoxicity assessment using the comet assay revealed a dose-dependent increase in DNA damage. Minimal DNA damage was observed at 25 µg/ml, while higher concentrations of 50 µg/ml and 100 µg/ml showed a progressive increase in DNA strand breaks. These findings align with prior studies demonstrating genotoxic effects caused by environmental and chemical stressors in biological systems. This study highlights the pharmacological potential of S. isoetifolium extract while emphasizing the importance of further investigations into its genotoxic effects at elevated concentrations. The results provide essential insights into the extract's bioactive components and safety profile, paving the way for future pharmacological and environmental toxicity research.

**Key words:** Marine; Seagrass; Ornamental fish; FTIR; Comet Assay

# Introduction

           Seagrasses play a crucial global role by supporting food security, mitigating climate change, enriching biodiversity, purifying water, protecting coastlines, controlling diseases [(Sarvesh et al., 2024)](https://paperpile.com/c/WqhHSd/TgyjO). Also beneficial from proximity to other coastal ecosystems like tidal marshes, coral reefs, mangrove forests, kelp forests, and oyster/mussel beds [(Heckwolf et al., 2021)](https://paperpile.com/c/WqhHSd/2Xwby). *Syringodium isoetifolium* meadows provide essential habitats for diverse marine organisms, offering food, shelter, and nursery grounds, thus enhancing local biodiversity [(Uku et al., 2021)](https://paperpile.com/c/WqhHSd/Ju34O). *Syringodium isoetifolium* is crucial for carbon sequestration, capturing and storing carbon dioxide, thereby aiding in climate change mitigation [(Bandh et al., 2023)](https://paperpile.com/c/WqhHSd/2mEWB). Also, they contribute significantly to global fisheries production, utilized for materials, medicines, and food resources, and also possess potential applications in biofuel, biogas, and biomethane production [(Apostoloumi et al., 2021)](https://paperpile.com/c/WqhHSd/MVOvH). They even contain a variety of bioactive compounds, such as phenolics, flavonoids, alkaloids, and terpenoids, which can have beneficial effects [(Vijai Selvaraj et al., 2025)](https://paperpile.com/c/WqhHSd/u2Oez). Also harmful effects on living organisms: likely the genotoxic effects of seagrass extracts on aquatic organisms involve the ability of chemical compounds extracted from seagrasses to induce damage to the genetic material (DNA) of these organisms [(Hernández-Balmaseda et al., 2021)](https://paperpile.com/c/WqhHSd/qPV87). The severity of genotoxic effects depends on factors such as the concentration and composition of the seagrass extracts, exposure duration, and the sensitivity of the fish species [(Schuijt et al., 2021)](https://paperpile.com/c/WqhHSd/XVzFc). When ornamental fishes are exposed to seagrass extracts, these compounds can interact with their DNA, leading to various forms of genetic damage [(Dubey et al., 2024)](https://paperpile.com/c/WqhHSd/u8Bu8). Genotoxic effects may include DNA strand breaks, formation of DNA adducts, induction of oxidative stress, chromosomal aberrations, and mutations[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/WqhHSd/gsk5+AhUZ+GVGZ). These genetic alterations can disrupt normal cellular functions, affect reproductive capacity, growth, and development, and ultimately impact the health and survival of the fishes [(Costa, 2022)](https://paperpile.com/c/WqhHSd/qhHdu). Such ornamental fish Betta splendens, also known as Siamese fighting fish is highly selective breeding for fighting ability, resulting in distinctive male aggression, as well as recent breeding for ornamental traits such as coloration, fin morphology, and body size, their unique characteristics and evolutionary lineage make them valuable models for research in behavior, endocrinology, neurobiology, genetics, development, and evolution [(Lichak et al., 2022)](https://paperpile.com/c/WqhHSd/41CIM). Male Betta fish are territorial and known for their aggressive behavior towards other males, leading to their nickname “fighting fish” Also, they flare their fins and display vivid colors when agitated or challenged by another male, which is why they are typically housed alone in aquariums unless with carefully selected tank mates [(Panthum et al., 2022)](https://paperpile.com/c/WqhHSd/HowYw). This study contributes to understand the potential ecological and genotoxic impacts of seagrass *Syringodium isoetifolium* derived compounds on ornamental fish Betta splendens[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/WqhHSd/1bhoy+yxugL+L1UAv)**.**

# Materials and methods

*Syringodium isoetifolium* seagrass was collected from Thondi Beach (latitude 9°44′15.6″N and longitude 78°02′05.9″E) in the Ramanathapuram district of Tamil Nadu and stored in a polythene bag. The species was identified by Dr. Pitchiah Sivaperumal and verified through manual identification. Voucher specimens were deposited at the Centre for Marine and Aquatic Research, The collected sample was thoroughly washed with water to remove sand, plastic debris, seashells, barnacles, rope fibers, and other impurities. The cleaned samples were then shallow-dried to eliminate excess moisture. Dried seagrass samples were coarsely ground to enhance the surface area for extraction. 20 grams of the powdered sample were mixed with 70% ethanol in a conical flask and placed on an orbital shaker for 48 hours. The mixture was agitated periodically to facilitate the extraction process. After 48 hours, the ethanolic extract was filtered using Whatman filter paper No. 1. The solvent was evaporated in a water bath at 60°C until a crude extract was obtained, which was then stored at 4°C for further analysis slightly modified by [(Kalaivani et al., 2023)](https://paperpile.com/c/WqhHSd/oZwAs).The phytochemical analysis of *Syringodium isoetifolium* was conducted to detect the presence of alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, and saponins. The results of the crude extract revealed the presence of various bioactive compounds, with detailed information provided in Table 1. The crude extract of the seagrass *Syringodium isoetifolium* was prepared for Fourier Transform Infrared (FT-IR) spectroscopy analysis. A sample of the crude extract was homogenized with dried potassium bromide (KBr) in a ratio of 1:99 (w/w). This mixture was then subjected to FT-IR scanning across a spectral range of 600 to 4000 cm⁻¹. The scanning was performed at a resolution of 1 micron per minute, utilizing a programmed slit opening, with air serving as the reference standard [(Shaffai et al., 2023)](https://paperpile.com/c/WqhHSd/Va3lI). Betta splendens were collected from the Kolathur fish market and maintained under standardized conditions with controlled temperature and humidity to ensure uniformity in size and health. Stock solutions of the test substance were prepared at concentrations of 25 μg/ml, 50 μg/ml, 75 μg/ml, and 100 μg/ml. A control group was included, using only 70% ethanol as a solvent to account for potential non-substance-related effects. Ten fish per concentration were individually transferred into tanks containing either the test solutions or the control solution, with replicates included for statistical analysis. The fish were then incubated under suitable environmental conditions, including controlled temperature, to assess DNA damage across different concentrations and the control group [(Heckwolf et al., 2021)](https://paperpile.com/c/WqhHSd/2Xwby).The comet assay was employed for the evaluation of DNA damage following the methodology previously described in[(Cui et al., 2021)](https://paperpile.com/c/WqhHSd/5Xhqd). This assay helps in the detection of low levels of DNA damage in individual cells, making it a valuable tool for environmental biomonitoring and toxicological studies in fish. To perform the comet assay, conventional microscope slides were dipped in molten regular agarose (1%, 60°C) for 1 minute, followed by drying the agarose layer at 50°C for 8 hours. A cell containing supernatant was prepared by homogenising the entire fish in 1 ml of PBS, from which a 10 μl aliquot was used for cell counting. Cells were then suspended in LMP agarose (0.7%, 37°C) at a 1:1 ratio with the cell-containing supernatant. 80 μl aliquot of this mixture was added onto the first agarose layer on the slides, which were chilled at 4°C for 10 minutes to form a second layer. Subsequently, another 80 μl of cell-free LMP agarose (0.7%, 37°C) was added, and the slides were chilled again at 4°C for 10 minutes to form a third layer. After solidification, the slides were placed in a lysis solution at 4°C for 2 hours. Following lysis, the slides were rinsed with ultrapure water to remove excess lysis solution and then incubated in an alkaline electrophoresis buffer at 4°C for 30 minutes to produce single-stranded DNA. Electrophoresis was conducted under alkaline conditions using the same buffer at 300 mA and 20 V for 20 minutes at 4°C. After electrophoresis, the slides were rinsed with ultrapure water and neutralized with two washes of neutralization buffer at room temperature for 10 minutes each. For DNA staining, 20 μl of propidium iodide (PI) solution was applied to the slides for 10 minutes. Finally, comet images were captured, and DNA damage was quantitatively analysed using a fluorescence microscope

# Results

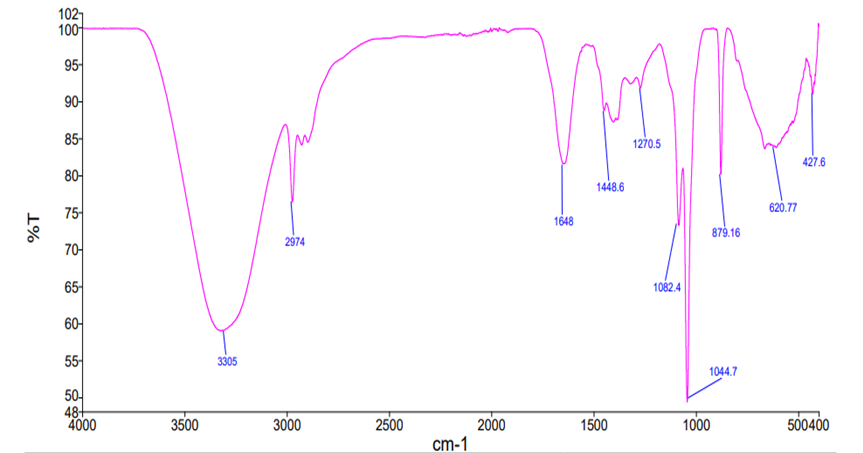
Phytochemical analysis of the ethanolic crude extract of *Syringodium isoetifolium* confirmed the presence of saponins, tannins, flavonoids, and alkaloids, as indicated by positive test results Table 1. In contrast, steroids and terpenoids were absent based on negative test findings. The presence of these bioactive compounds suggests the extract's potential pharmacological properties, typically attributed to saponins, tannins, flavonoids, and alkaloids.

**Table 1.** Phytochemical analysis of crude extract

|  |  |  |
| --- | --- | --- |
| S.No | TEST | RESULTS |
| 1. | Saponins | + |
| 2. | Tannins | + |
| 3. | Steroids | - |
| 4. | Flavonoids | + |
| 5. | Terpenoids | - |
| 6. | Alkaloids | + |

The Fourier-transform infrared (FTIR) spectrum Figure1. Showed the characteristic absorption peaks indicative of different functional groups that are present in the sample. The broad absorption peak at 3305 cm⁻¹ indicates O-H stretching vibrations, commonly associated with alcohols and phenols (Almatrafi et al., 2024). The peak at 2974 cm⁻¹ corresponds to C-H stretching vibrations, characteristic of alkanes. The strong absorption band at 1648 cm⁻¹ indicates C=O stretching vibrations, which can be attributed to carbonyl compounds such as aldehydes, ketones, carboxylic acids, or esters(Saadh et al., 2024). The peak at 1448.6 cm⁻¹ corresponds to C-H bending vibrations, commonly found in alkanes and aromatic compounds. The absorption band at 1270.5 cm⁻¹ suggests the presence of C-O stretching vibrations, characteristics of alcohols, ethers, carboxylic acids, and esters. Peaks at 1082.4 cm⁻¹ and 1044.7 cm⁻¹ are characteristic of C-O-C stretching vibrations, suggesting the presence of ethers. The absorption at 879.16 cm⁻¹ corresponds to C-H out-of-plane bending in aromatic compounds, while the peaks at 620.77 cm⁻¹ and 427.6 cm⁻¹ are associated with skeletal vibrations of the molecule.

The comet test was used to evaluate the genotoxic effects of crude extract from Syringodium isoetifolium on Betta splendens. Figure 2.A) the control group shown in the figure has round, undamaged nuclei that show that there is no DNA damage. In contrast, the following image, which represents the maximum concentration (100 μg/ml), exhibits considerable DNA strand breaks and widespread DNA fragmentation, as seen by a dispersed structure with pronounced tail development. A dose-dependent rise in DNA damage at various concentrations is shown in the graph. DNA damage is insignificant (2%) at the lowest concentration (25μg/ml), indicating a negligible genotoxic effect. With comet tail development of 10–15 μm and DNA damage levels increasing to 6–10%, DNA damage becomes more noticeable as the concentration increases to 50 μg/ml. Moderate damage and increased comet tail elongation are seen at 75 μg/ml. Significant genotoxic stress on the cells is indicated by the severe DNA fragmentation at the highest dose (100 μg/ml), Figure 3 and Figure 2.B) with tail length surpassing 20 μm and DNA damage peaking at ~14–16%. To support a dose-dependent oxidative stress response and confirm the genotoxic potential of Syringodium isoetifolium, the most common forms of DNA damage are class II (moderate tail creation) at lower concentrations and types III-IV (extensive fragmentation) at higher concentrations.

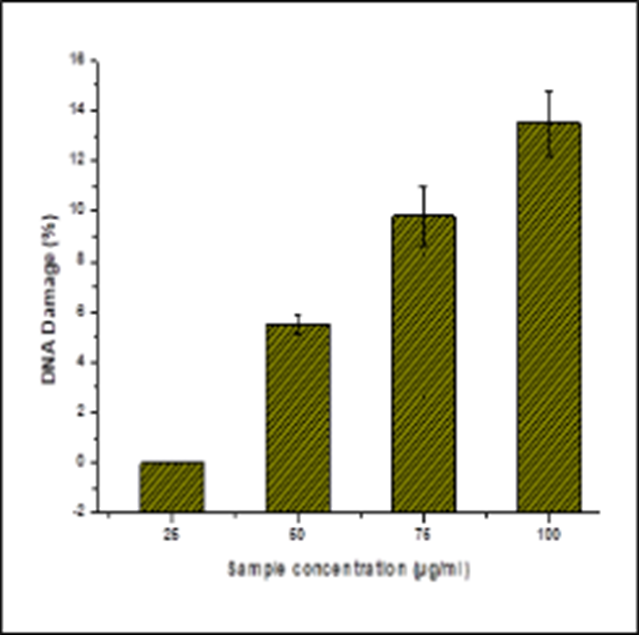


**Figure 1**. FTIR Analysis of crude extract from seagrass *Syringodium isoetifolium*

1. **(B)**

**Figure 2**.(A) Control group (B) DNA damage at higher concentration by Fluorescence microscopic analysis DNA damage of Siamese fighting fish using crude extract of Syringodium isoetifolium

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**Figure 3.** Representation of DNA damage through percentage using different concentration of crude extract

# Discussions

In 2020, [(Monisha et al., 2020)](https://paperpile.com/c/WqhHSd/yC6Zj) observed the FTIR analysis of *Enhalus acoroides* seagrass identified various functional groups[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/WqhHSd/WzwaN+wtDyr+qWST5). The symmetric C-H stretching vibrations for alkenes and alkanes were detected at 884 cm⁻¹ and 2969 cm⁻¹, respectively. Vibrational modes associated with the –CH₂– group were observed at 2848 cm⁻¹ (symmetric), 2916 cm⁻¹ (asymmetric), and 1462 cm⁻¹ (scissoring). Aromatic C-H bending vibrations appeared around 718 cm⁻¹. Peaks at 1027 cm⁻¹ and 1710 cm⁻¹ indicated C-O and C=O stretching vibrations, suggesting the presence of carbonyl compounds, alcohols, carboxylic acids, and esters. Stretching of C=C bonds in alkenes and conjugated systems was observed at 1644 cm⁻¹ and 1576 cm⁻¹[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/WqhHSd/6DkaT+LfzWq+Vy5LD). Bands at 1239 cm⁻¹ and 1378 cm⁻¹ were attributed to C-N and N-O stretching vibrations in amines/amides and nitro compounds, respectively. A broad peak around 3344 cm⁻¹ was due to the stretching vibration of alcoholic groups, particularly phenols. These functional group vibrations indicate the presence of flavonoids, alkaloids, terpenoids, and phenols in *E. acoroides* seagrass. In 2024,[(Mohamed et al., 2024)](https://paperpile.com/c/WqhHSd/QQQmz) FTIR spectrum of *Thalassia hemprichii* seagrass identified the presence of several functional groups, including alkanes, cyclic alcohols, aromatic amine III, hydroxyl, amide, and ketone groups, indicative of an abundance of terpenoids and flavonoids. The symmetric and asymmetric stretching vibrations of methylene (-CH₂) were detected at 2850 cm⁻¹ and 2919 cm⁻¹, respectively. Peaks at 1722 cm⁻¹ and 1380 cm⁻¹ indicated C=O and C-H stretching, while a peak at 1460 cm⁻¹ corresponded to the asymmetric bending vibration (δ) of C-H and the aromatic stretching vibrations associated with CH₃ and CH₂ groups in flavonoids and aromatic rings. A broad, strong band at 3261 cm⁻¹ and 3304 cm⁻¹ suggested OH-stretching vibrations typical of phenols or alcohols, which could also imply the presence of lipids. C-N stretching bands indicated the presence of amines, amides, and proteins. Additionally, a C=C stretching vibration ring at 1409.43 cm⁻¹ was attributed to flavonoids and amino acids.The comet assay results reported by [(Obiakor et al., 2021)](https://paperpile.com/c/WqhHSd/aX1p3) indicate significant DNA migration in erythrocyte cells of silver perch following exposure to Sb (III), characterized by a distinct comet-like appearance, signifying DNA damage[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/WqhHSd/ZOsj4+kIdzI+g9QHs)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/WqhHSd/ZOsj4+kIdzI+g9QHs+o9eoZ). Exposure to Sb (V) also resulted in comet-like structures, though with varying tail lengths and intensities, suggesting different levels of DNA fragmentation. In contrast, the control group exhibited minimal to no DNA migration, indicating intact DNA with no significant damage[(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi & 2021)](https://paperpile.com/c/WqhHSd/ca8ma+jiWfE+Aybtl)[(Pranati et al., 2021; Sakthi et al., 2021)](https://paperpile.com/c/WqhHSd/ca8ma+jiWfE)[(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/WqhHSd/ca8ma+jiWfE+Aybtl)). These findings confirm that both Sb (III) and Sb (V) induce DNA damage in erythrocyte cells, with Sb (III) exerting more pronounced effects. Furthermore, as discussed by [(Khan et al., 2023)](https://paperpile.com/c/WqhHSd/IrDhw), the frequency of comet-positive cells followed a dose-dependent pattern concerning nanoparticle concentration. At lower concentrations (10–30 mg/L), erythrocytes showed slight DNA damage [(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/WqhHSd/mzduQ+fXf3A)). However, as nanoparticle concentrations increased, the extent of DNA fragmentation also escalated. Initially, cellular DNA repair mechanisms managed to counteract slight to moderate damage. However, at higher concentrations and prolonged exposure (28 days), these repair systems became overwhelmed, leading to severe and persistent DNA damage.

# Conclusion

These findings highlight the dual nature of *Syringodium isoetifolium* extract while it contains bioactive compounds with pharmacological potential, its genotoxic effects at higher concentrations raise concerns about environmental toxicity and safety. Further studies are needed to explore the mechanisms of genotoxicity, safe dosage levels, and potential applications of this seagrass extract in both biomedical and environmental contexts. This research provides valuable insights into the bioactive properties and toxicological impact of *S. isoetifolium*, contributing to future studies on marine-derived compounds and their ecological implications.

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