RESEARCH PAPER

Comparative Evaluation of the Antibacterial and Preservative Potential of *Halophila Ovalis* and *Syringodium Isoetifolium* Ethanolic Extracts in Chicken Meat

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Abstract

Seagrasses are promising sources of bioactive compounds with potential applications as natural antimicrobial agents and food preservatives. The present study comparatively evaluated the antibacterial and preservative potential of ethanolic extracts of *Halophila ovalis* and *Syringodium isoetifolium* in chicken meat. Bacterial isolates associated with the seagrasses were characterized using phenotypic, biochemical and molecular approaches. The antibacterial activity of the extracts was evaluated by disc diffusion and agar well diffusion assays, followed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Both extracts exhibited antibacterial activity, with *S. isoetifolium* showing comparatively greater inhibition than *H. ovalis*. MIC and MBC assays further supported the inhibitory and bactericidal effects of the extracts. Application of the extracts to chicken meat during refrigerated storage resulted in slower deterioration compared with untreated samples, with *S. isoetifolium* demonstrating better preservation performance. GC–MS analysis revealed diverse constituents, including fatty acids, alcohols and phenolic derivatives, while FTIR analysis indicated the presence of multiple functional groups. Overall, the findings demonstrate the potential of *H. ovalis* and *S. isoetifolium* extracts as natural antimicrobial preservatives, with *S. isoetifolium* showing greater promise for application in chicken meat preservation.

Keywords: Seagrass; antibacterial activity; natural preservative; chicken meat; MIC; MBC; GC–MS; FTIR.

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1. Introduction

Microbial spoilage remains one of the major challenges affecting the quality and shelf stability of perishable foods. Fresh poultry products are particularly susceptible to microbial deterioration because of their high moisture content, nutrient availability and relatively short refrigerated shelf life. Consequently, effective preservation strategies are required to control microbial growth while maintaining food quality and safety. Although synthetic preservatives and other chemical interventions are widely used, increasing interest in naturally derived alternatives has stimulated research into plant- and marine-derived antimicrobial compounds. Natural antimicrobial and antioxidant compounds are being investigated for their potential application in food preservation because of their diverse biological activities and possible incorporation into different food systems (Bensid et al., 2022).

Marine organisms represent an important and comparatively underexplored source of bioactive metabolites. Marine plants, including seagrasses, are exposed to complex environmental conditions and possess diverse secondary metabolites that may contribute to ecological interactions and defence. Chemical investigations have identified numerous classes of secondary metabolites from seagrasses, including phenolic derivatives, flavonoids, steroids and

other structurally diverse compounds, with reported antimicrobial and other biological activities (Jensen et al., 2016). More recent reviews have likewise highlighted seagrasses as promising sources of bioactive molecules with potential applications extending beyond their established ecological importance (Ganesan et al., 2024).

The antimicrobial potential of marine-derived natural products has particular relevance to food preservation. Research on marine bioresources has demonstrated that naturally occurring compounds can inhibit food-associated microorganisms and may be incorporated into preservation approaches such as direct application, edible coatings and active packaging. However, the activity of a natural extract depends on several factors, including the biological source, extraction solvent, concentration and composition of the resulting preparation (Pina-Pérez et al., 2017; Surendhiran et al., 2021). These considerations support the need for species-specific evaluation of marine plant extracts under relevant food conditions rather than assuming uniform antimicrobial activity across different sources.

Among seagrasses, *Halophila ovalis* and *Syringodium isoetifolium* are distributed in tropical and subtropical marine environments and have been investigated for their chemical and biological properties. Previous work on *H. ovalis* has demonstrated antibacterial activity of its extracts and identified diverse chemical constituents, including lipid-associated compounds and phenolic constituents (Yuvaraj et al., 2012). Investigations of *S. isoetifolium* have likewise demonstrated the presence of biologically active metabolites, while comparative studies of Indian seagrasses have shown that phytochemical composition can vary considerably among species (Kannan et al., 2013). Such species-dependent variation provides a rationale for directly comparing different seagrasses when assessing their potential as sources of natural antimicrobials.

Chemical characterization is an important component of evaluating natural extracts because the biological activity of a complex extract may arise from several constituents acting individually or in combination. GC–MS provides a means of profiling detectable chemical constituents, whereas FTIR provides information regarding characteristic functional groups within an extract. These complementary approaches can assist in relating the chemical characteristics of marine plant extracts to their observed biological properties.

Despite increasing interest in marine-derived antimicrobials, the application of seagrass extracts directly to food matrices remains comparatively less explored than their general antimicrobial or pharmacological evaluation. In particular, comparative assessment of *H. ovalis* and *S. isoetifolium* extracts in a poultry matrix can provide useful information on whether there in vitro antibacterial properties translate into preservation potential under refrigerated storage conditions.

Therefore, the present study was undertaken to compare the antibacterial and preservative potential of ethanolic extracts of *H. ovalis* and *S. isoetifolium*. The study evaluated their antibacterial activity using diffusion assays, determined their minimum inhibitory and bactericidal concentrations, assessed their preservative effect on chicken blocks during refrigerated storage and characterized the extracts using GC–MS and FTIR. The comparative approach was intended to identify the relative potential of the two seagrass extracts as natural antimicrobial agents for application in food preservation.

2. Materials and Methods

2.1 Collection and identification of seagrasses

Halophila ovalis and *Syringodium isoetifolium* were collected from Pulicat Lake, Tamil Nadu, during August 2025 (Figure 1). The samples were collected in polyethylene bags containing seawater to prevent desiccation and transported to the laboratory under aseptic conditions on the day of collection. Preliminary identification was based on morphological characteristics including leaf morphology, arrangement and growth pattern. The collected specimens were subsequently processed for further analysis.



Fig. 1a: Syringodium isoetifolium



Fig. 1b: Halophila ovalis

2.2 Processing and preparation of seagrass powder

The collected seagrass leaves were thoroughly washed with sterile distilled water to remove adhering sand, epiphytes and other debris. The cleaned leaves were transferred to a sterile mortar and homogenized using a pestle. For extract preparation, the samples were shade-dried at room temperature for 5–7 days until complete removal of moisture. The dried material was cut into small pieces and ground into a fine powder using a sterile mechanical grinder. The powdered samples were stored in sterile airtight containers at room temperature until extraction.

2.3 Preparation of ethanolic extracts

Approximately 10 g of dried powder from each seagrass species was extracted with 100 mL ethanol under constant agitation for 48 h. Following extraction, the mixtures were filtered through Whatman filter paper. The resulting filtrates were concentrated using a rotary evaporator to remove the solvent and obtain crude ethanolic extracts. The extracts were stored at 4 °C until further antimicrobial and preservation studies.

2.4 Isolation of bacteria associated with the seagrasses

For isolation of bacteria associated with the collected seagrasses, homogenized samples were inoculated onto Nutrient Agar and Zobell agar media. The inoculated plates were incubated at room temperature for 24 h. Representative colonies exhibiting distinct morphological characteristics were selected and subcultured on fresh agar media to obtain pure cultures. The purified isolates were maintained on Nutrient Agar slants for subsequent identification and antimicrobial studies.

2.5 Preliminary identification of bacterial isolates

The bacterial isolates were initially characterized based on colony morphology and microscopic examination, followed by biochemical characterization which includes Gram staining, catalase, motility, oxidative/fermentative reaction, methyl red, Voges–Proskauer, indole, citrate, ONPG and nitrate reactions, followed by carbohydrate fermentation tests.

2.6 Molecular identification of bacterial isolates

Selected bacterial isolates were subjected to molecular identification using 16S rRNA gene analysis. Genomic DNA was extracted from selected isolates and amplified using universal primers, **hemolysin (*hly*)** and **aerolysin (*aer*)** markers, with amplicon sizes of 597 bp and 416 bp, respectively, followed by molecular identification.

2.7 Antibacterial activity of seagrass extracts

2.7.1 Disc diffusion assay

The antibacterial activity of the ethanolic extracts of *H. ovalis* and *S. isoetifolium* was evaluated against the test bacterial cultures (*Staphylococcus aureus* and *Escherichia coli*) using the disc diffusion method.

Test cultures were grown in Nutrient Broth at 37 °C for 18–24 h. Mueller–Hinton Agar plates were prepared and bacterial suspensions were uniformly inoculated over the agar surface using sterile cotton swabs to obtain confluent growth. Sterile filter-paper discs impregnated with different concentrations of the seagrass extracts were placed on the inoculated agar surface using sterile forceps. A solvent-only disc was included as a control. The plates were incubated at 37 °C for 24 h, after which the diameter of the inhibition zones surrounding the discs was measured.

2.7.2 Agar well diffusion assay

Antibacterial activity was additionally evaluated using the agar well diffusion method. Bacterial cultures were uniformly inoculated onto the surface of the appropriate agar medium using a sterile swab. Wells of uniform diameter were prepared using a sterile cork borer and the seagrass extracts were introduced into the wells at volumes of 25, 50, 75 and 100 µL. A solvent-only well served as the control. Following incubation at 37 °C for 24 h, antibacterial activity was evaluated by measuring the diameter of the zones of inhibition.

2.8 Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the ethanolic extracts was determined by the broth dilution method. Fresh bacterial cultures were prepared in Nutrient Broth and incubated at 37 °C for 18–24 h. Serial concentrations of the seagrass extracts ranging from 128 to 0.25 mg/L were prepared in Nutrient Broth. Each concentration was inoculated with standardized bacterial suspension. An extract-free inoculated tube served as the positive growth control, while a tube containing uninoculated broth served as the negative control. The tubes were incubated at 37 °C for 24 h, and the MIC was recorded as the lowest extract concentration showing no visible bacterial growth.

2.9 Determination of minimum bactericidal concentration

MBC was determined from the tubes that showed no visible growth in the MIC assay. A loopful of culture from the growth-negative tubes was streaked onto fresh Nutrient Agar plates and incubated at 37 °C for 24 h. The MBC was considered the lowest concentration at which no bacterial growth was observed following subculture.

2.10 Preparation and treatment of chicken samples

Fresh chicken samples were obtained from a local hygienic market and transported to the laboratory under sterile conditions. The samples were washed with sterile distilled water, excess moisture was removed using

sterile blotting paper, and the meat was cut into uniformly sized blocks using a sterile knife.

The chicken blocks were divided into experimental groups and immersed in ethanolic extracts of *H. ovalis* and *S. isoetifolium* at concentrations of 1%, 2% and 3% (w/v). The treated samples were allowed to soak for a specified period to facilitate contact with the extracts. Treated and untreated control samples were subsequently stored under refrigerated conditions for evaluation of preservative activity.

2.11 Evaluation of preservative activity during storage

The preservative effect of the seagrass extracts was evaluated by monitoring microbial growth in treated and untreated chicken samples during refrigerated storage. Samples were analyzed at predetermined storage intervals (days 0, 3, 5, 7 and 10). A known quantity of chicken from each treatment was aseptically collected and homogenized in sterile saline. Serial dilutions were prepared, and appropriate dilutions were plated onto Nutrient Agar using the spread-plate plated onto Nutrient Agar using the spread-plate technique. Plates were incubated at 37 °C for 24 h, after which colonies were enumerated. Microbial load was expressed as CFU/g of chicken sample.

2.12 FTIR analysis

The seagrass extracts were characterized using Fourier-transform infrared spectroscopy (FTIR) to identify major functional groups present in the extracts.

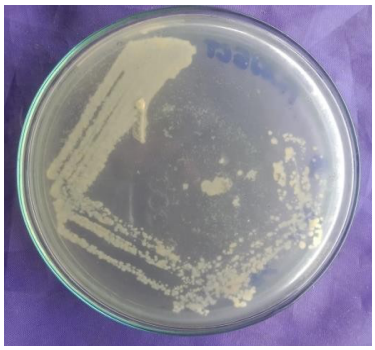
2.13 GC–MS analysis

The extracts were subjected to GC–MS analysis for characterization of their chemical constituents such as fatty acids, alcohols phenolic derivatives, β-sitosterol, salicylamide and salicylic acid.

3. Results

3.1 Processing and Isolation of Seagrass-Associated Bacteria

The *Halophila ovalis* and *Syringodium isoetifolium* samples were processed and bacterial isolates associated with the seagrasses were obtained by cultivation on Zobell agar and Nutrient agar. Eight bacterial isolates were recovered and designated as HO-1, HO-2, HO-3 and HO-4 from *H. ovalis*, and SI-1, SI-2, SI-3 and SI-4 from *S. isoetifolium*. Representative growth of the bacterial isolates on Zobell and nutrient agar is shown in Figures 2 and 3.



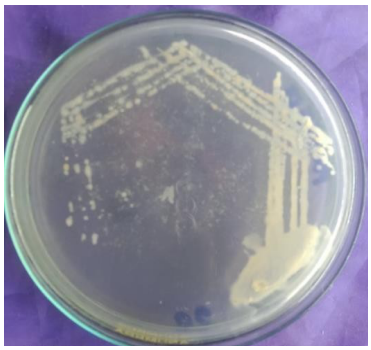


Fig. 2: Bacterial isolates on Zobell medium

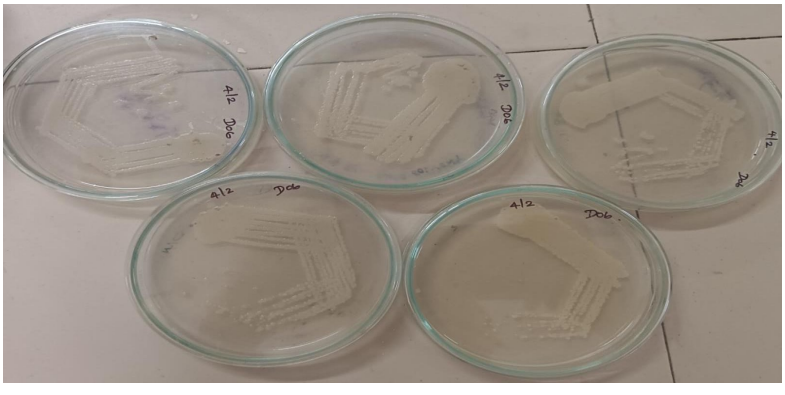
Fig. 3: Bacterial isolates on Nutrient agar medium

3.2 Phenotypic and Biochemical Characterization of the Isolates

The colony morphology of the isolates differed with respect to colony shape, size, colour, margin, elevation, texture and opacity. The *H. ovalis*-associated isolates included circular and irregular colonies ranging from small to large, with creamy white, pale yellow or white coloration. The *S. isoetifolium*-associated isolates similarly exhibited variation in colony morphology, including circular and irregular colonies with creamy white, yellowish and white coloration.

All isolates were recorded as Gram-negative and catalase positive. The *H. ovalis* isolates were motile, whereas the *S. isoetifolium* isolates were recorded as non-motile. Both groups showed a fermentative reaction in the O/F test. Differences were observed in methyl red, indole and citrate reactions, while both groups were positive for Voges–Proskauer, ONPG and nitrate reduction.

Carbohydrate fermentation testing showed acid and gas



production with glucose and lactose among both groups. Sucrose and maltose were fermented with acid and gas production by the *H. ovalis* isolates, whereas acid production was recorded for the *S. isoetifolium* isolates. Mannitol produced an acid reaction in the *H. ovalis* group and an acid and gas reaction in the *S. isoetifolium* group.

3.3 Molecular Characterization of the Isolates

The bacterial isolates were subjected to molecular characterization using 16S rRNA gene analysis. In addition, PCR-based detection of selected virulence-associated markers was reported. Isolates associated with *H. ovalis* showed a distinct 597-bp amplicon corresponding to the *hly* gene. The isolates associated with *S. isoetifolium* produced a 416-bp amplicon corresponding to the *aer* gene. Based on these markers, the isolates were reported as putatively belonging to the genera *Vibrio* and *Aeromonas*, respectively.

3.4 Antibacterial Activity by Disc Diffusion

The ethanolic extracts of both *H. ovalis* and *S. isoetifolium* exhibited antibacterial activity against the tested bacterial cultures, as indicated by the formation of clear zones of inhibition around the extract-impregnated discs. The inhibition zones observed with the *S. isoetifolium* extract were comparatively larger than those observed with the *H. ovalis* extract, indicating greater antibacterial activity of the former.

The solvent control discs did not produce visible zones of inhibition. Representative inhibition zones for both extracts and the solvent control are shown in Figure 4.

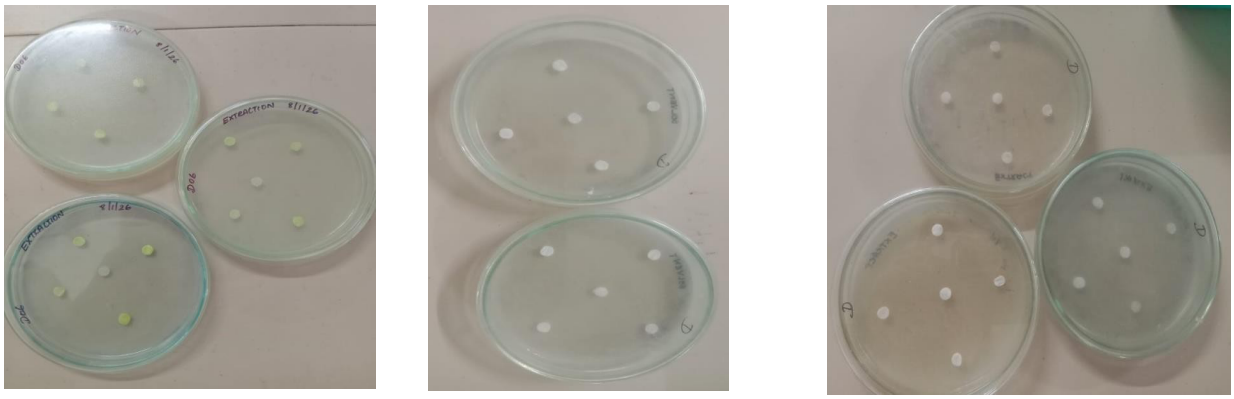


Fig. 4: (a) Zone of inhibition of Halophila ovalis, (b) Solvent, (c) Zone of inhibition of Syringodium isoetifolium

3.5 Antibacterial Activity by Agar Well Diffusion

Agar well diffusion analysis similarly demonstrated inhibitory activity for both seagrass extracts. Clear zones were observed surrounding the wells containing the extracts. The *S. isoetifolium* extract produced comparatively larger inhibition zones than the *H. ovalis* extract, consistent with the observations obtained using the disc diffusion assay. No inhibition was observed around the control well. Representative results are shown in Figures 5 and 6.

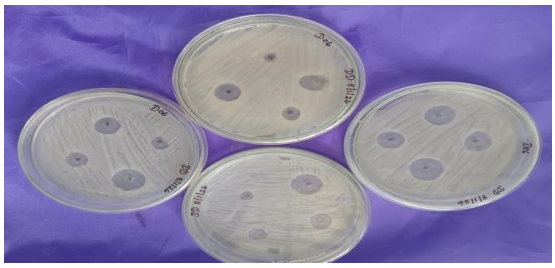


Fig. 5: Zone of inhibition by Syringodium isoetifolium extract

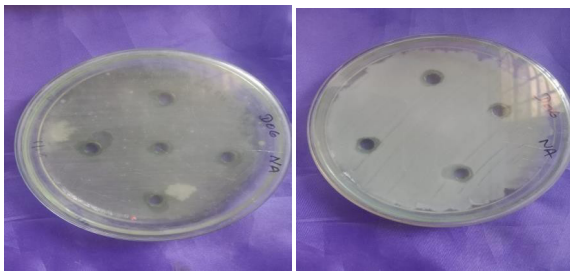


Fig. 6: Zone of inhibition by Halophila ovalis extract

3.6 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the two ethanolic seagrass extracts was determined by broth dilution. Both extracts demonstrated inhibitory activity against the tested bacterial isolates. The *S. isoetifolium* extract exhibited a lower MIC than the *H. ovalis* extract. The absence of visible turbidity in the corresponding MIC tubes indicated inhibition of bacterial growth. Representative MIC observations for the two extracts are shown in Figure 7.

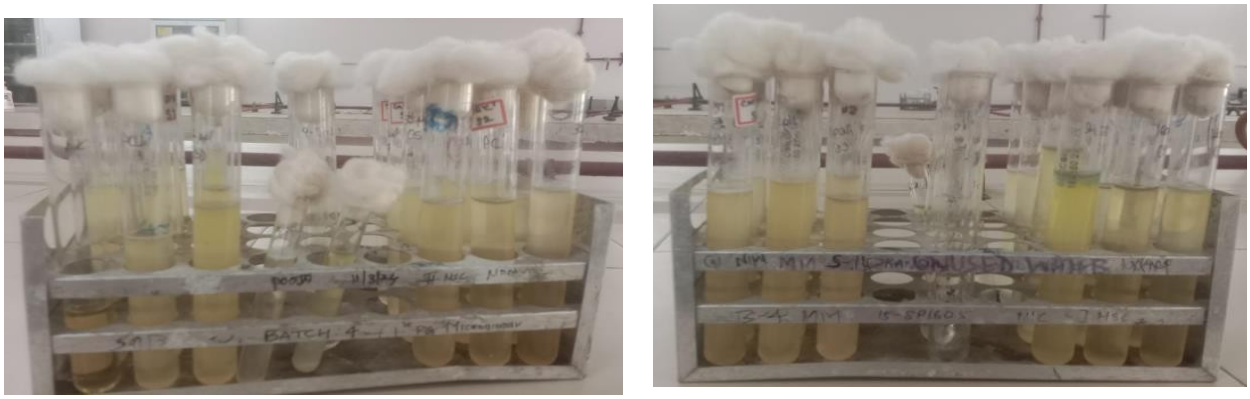


Fig. 7: (a) MIC tubes containing Halophila ovalis seagrass extract and (b) MIC tubes containing Syringodium isoetifolium seagrass extract

3.7 Minimum Bactericidal Concentration

Both seagrass extracts exhibited bactericidal activity against the tested isolates. The MBC values were reported to be slightly higher than the corresponding MIC values. Among the two extracts, *S. isoetifolium* demonstrated comparatively greater bactericidal activity than *H. ovalis*. The MBC observations are shown in Figure 8.

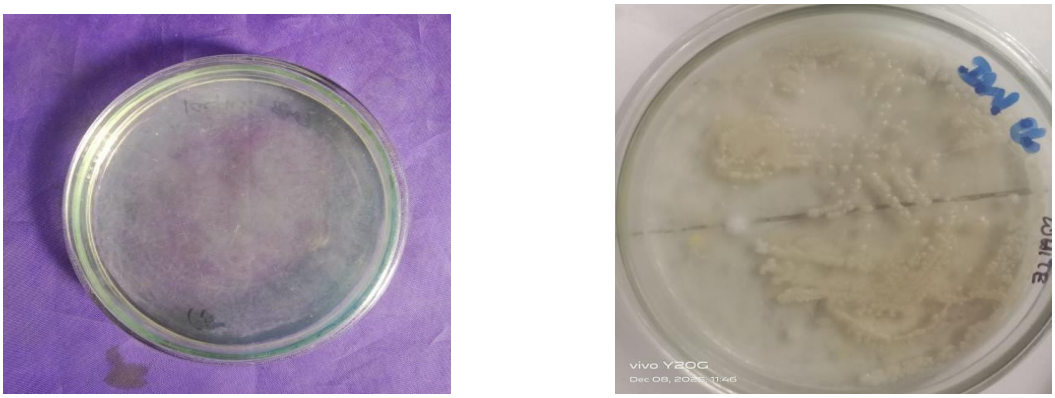


Fig. 8: (a) MBC plates containing Syringodium isoetifolium seagrass extract and (b) MBC plates containing Halophila ovalis seagrass extract

3.8 Preservative Effect of Seagrass Extracts on Chicken

The preservative potential of the seagrass extracts was evaluated using chicken blocks treated with *H. ovalis* and *S. isoetifolium* extracts and maintained under refrigerated storage. Changes in microbial load and visible spoilage characteristics, including colour, texture and odour, were monitored during storage. The untreated control samples showed a more rapid increase in microbial growth accompanied by changes in colour, texture and odour. In comparison, the extract-treated samples showed a slower increase in microbial load. Among the two extracts, *S. isoetifolium* showed better preservation efficiency than *H. ovalis*. Representative chicken samples at days 0, 3, 5, 7 and 10 are shown in Figures 9, 10, 11, 12 and 13.



Fig. 9: Chicken blocks coated with seagrass extracts of Halophila ovalis and Syringodium isoetifolium (Day 0)



Fig. 10: Chicken blocks coated with seagrass extracts of Halophila ovalis and Syringodium isoetifolium (Day 3)



Fig. 11: Chicken blocks coated with seagrass extracts of Halophila ovalis and Syringodium isoetifolium (Day 5)



Fig. 12: Chicken blocks coated with seagrass extracts of Halophila ovalis and Syringodium isoetifolium (Day 7)



Fig. 13: Chicken blocks coated with seagrass extracts of Halophila ovalis and Syringodium isoetifolium (Day 10)

3.9 GC–MS Analysis

GC–MS analysis of the seagrass extracts indicated the presence of various bioactive compounds, with fatty acids, alcohols and phenolic derivatives reported among the major compound classes. Method repeatability was assessed at the limit of quantitation, with percentage relative standard deviation (%RSD) values of 0.6% for

Sample A and 2.5% for Sample B, both within the reported acceptance criterion of ≤15%.

Recovery analysis showed average recovery values of 96.7% for Sample A and 92.8% for Sample B, which were within the reported acceptance range of 70–130%.

The chromatographic analysis also indicated the presence of β-sitosterol, along with markers corresponding to salicylamide and salicylic acid (Figure 4).

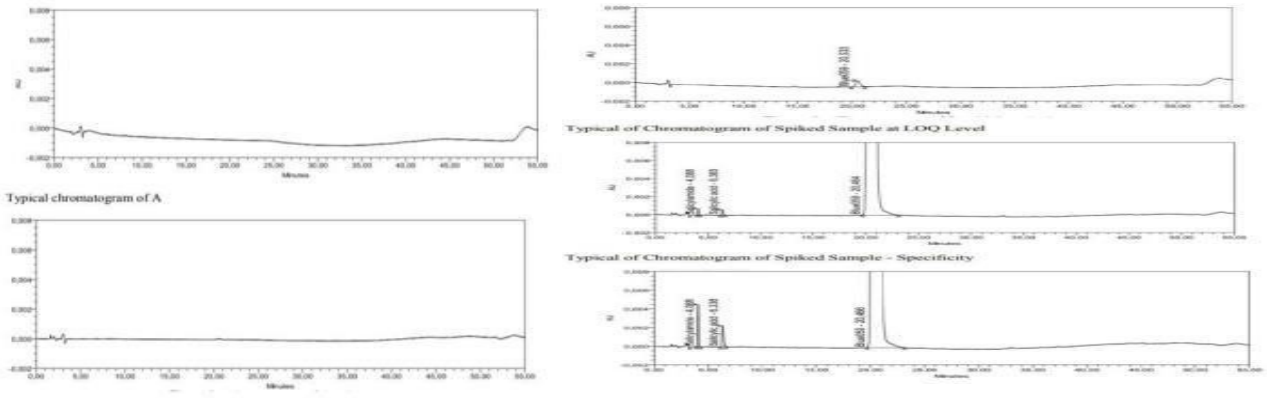


Fig. 14: GC-MS spike analysis

3.10 FTIR Analysis

FTIR analysis demonstrated the presence of several functional groups in the seagrass extracts. Major absorption peaks were observed at 3740.03, 3281.04, 2903.30, 1612.69, 1402.96, 1242.33, 1023.12, 658.19 and 582.88 cm^{-1}. These peaks were reported to correspond to hydroxyl groups, alcohols, carboxylic acids, aromatic compounds, nitro compounds, alkyl halides and aryl halides.

The FTIR profile was further reported to indicate the presence of phytochemical constituents including phenols, flavonoids and tannins. The FTIR spectrum of the seagrass extracts is presented in Figure 15.

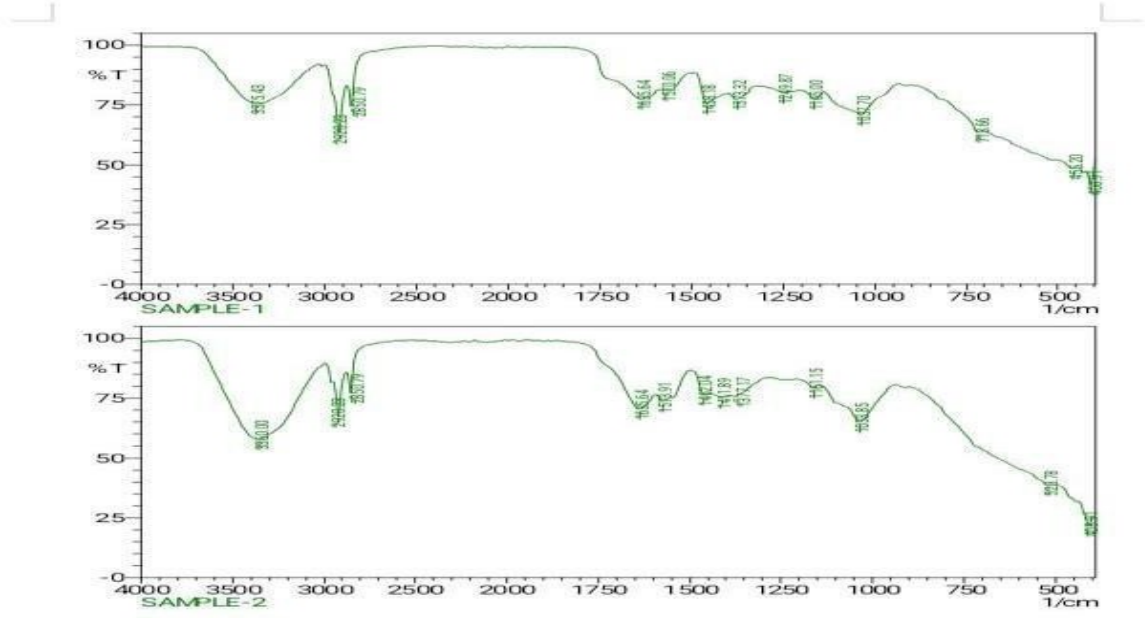


Fig. 15: FTIR analysis of seagrass extracts

4. Discussion

The present study demonstrated that ethanolic extracts of *Halophila ovalis* and *Syringodium isoetifolium* possess antibacterial activity, with *S. isoetifolium* exhibiting comparatively greater activity. Both extracts produced inhibition zones in disc diffusion and agar well diffusion assays, whereas the solvent controls showed no inhibition. Similar antibacterial activity has been reported among Indian seagrasses, with chemical profiling demonstrating diverse bioactive metabolites (Kannan et al., 2012). The greater activity of *S. isoetifolium* observed in the present study is consistent with previous reports. Iyapparaj et al. (2014) demonstrated antimicrobial activity of *S. isoetifolium* extracts and identified fatty acids and their esters among the major metabolites. Sathyanathan et al. (2016) further identified pheophytin a from *S. isoetifolium* and demonstrated antibacterial activity against *Salmonella Typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The antibacterial activity of *H. ovalis* is also supported by previous findings. Yuvaraj et al. (2012) reported antibacterial activity of a methanolic extract of *H. ovalis* against Gram-positive and Gram-negative bacteria and identified several chemical constituents by GC–MS.

Although the extraction solvent differed from that used in the present study, the findings support the antimicrobial potential of this species. Differences in activity between the two seagrasses may therefore be related to variation in the composition and abundance of bioactive constituents.

The MIC and MBC findings further supported the antibacterial activity observed in the diffusion assays. Both extracts exhibited inhibitory and bactericidal effects, with *S. isoetifolium* showing comparatively greater effectiveness. The consistency of these findings across diffusion and broth-based assays strengthens the evidence for the antibacterial potential of the extracts.

The bacterial isolates recovered from the two seagrasses exhibited distinct phenotypic and biochemical characteristics. Molecular analysis detected a 597-bp *hly* amplicon among isolates associated with *H. ovalis* and a 416-bp *aer* amplicon among isolates associated with *S. isoetifolium*. These findings indicate the presence of isolates carrying the respective marker genes and complement the phenotypic characterization of the isolates.

The application of the extracts to chicken meat demonstrated their preservative potential. Untreated samples showed more rapid deterioration, including changes in colour, texture and odour, whereas extract-treated samples exhibited slower spoilage. *S. isoetifolium* showed better preservation performance than *H. ovalis*, consistent with its stronger antibacterial activity in vitro. Similar effects of natural antimicrobial treatments on chicken products have been reported, with natural compounds reducing the growth of spoilage-associated microorganisms during refrigerated storage (Osaili et al., 2024).

GC–MS analysis revealed fatty acids, alcohols and phenolic derivatives, together with β-sitosterol and compounds corresponding to salicylamide and salicylic acid. The presence of diverse chemical constituents may contribute to the observed antibacterial activity. Previous GC–MS studies of Indian seagrasses have likewise demonstrated chemically diverse metabolites associated with biological activity (Kannan et al., 2012; Iyapparaj et al., 2014). FTIR analysis further supported the presence of diverse functional groups, including those associated with hydroxyl-containing compounds, alcohols, carboxylic acids and aromatic structures. Together, the GC–MS and FTIR findings suggest that the biological activity of the extracts may result from the combined contribution of multiple constituents rather than a single compound.

Overall, both *H. ovalis* and *S. isoetifolium* demonstrated antibacterial and preservative potential, with *S. isoetifolium* consistently showing comparatively greater effectiveness. The agreement between the antimicrobial assays, chicken preservation observations and chemical characterization supports the potential of *S. isoetifolium* as a source of natural antimicrobial compounds for food-preservation applications.

5. Conclusion

The present study demonstrated that ethanolic extracts of *Halophila ovalis* and *Syringodium isoetifolium* possess antibacterial activity and preservative potential. Both extracts showed inhibitory activity in disc diffusion and agar well diffusion assays, while MIC and MBC assessments further supported their inhibitory and bactericidal effects. Among the two seagrasses, *S. isoetifolium* consistently exhibited comparatively greater antibacterial activity.

Application of the extracts to chicken blocks demonstrated their potential to delay microbial deterioration during refrigerated storage. The treated samples showed slower spoilage compared with the untreated control, with *S. isoetifolium* providing better preservation performance than *H. ovalis*.

GC–MS analysis revealed diverse chemical constituents, including fatty acids, alcohols and phenolic derivatives, while FTIR analysis demonstrated functional groups associated with several classes of bioactive compounds. These findings provide a chemical basis for the biological activity observed in the extracts. The combined microbiological and chemical findings indicate that the antibacterial activity may result from the collective action of multiple constituents rather than a single compound.

Overall, *S. isoetifolium* showed greater promise than *H. ovalis* as a natural antimicrobial and preservative agent for chicken. The findings support further investigation

of seagrass-derived bioactive compounds for potential application in natural food-preservation systems.

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