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ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF SEAWEED (*ENTEROMORPHA INTESTINALIS*) AGAINST JUICE-DERIVED PATHOGENS

Vijay krishnan[*1], Gokul Surendra kumar G[2], Balaji.B[3], and Geetha, C[4]

[*1]Peri college of physiotherapy, Chennai -48

[2 &3] Peri Institute of Technology, Chennai - 48

[4] Peri college of Nursing, Chennai -48

Corresponding mail id: publications@peri.ac.in

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ABSTRACT

This study investigates the in vitro antimicrobial efficacy of 90% methanolic extract of *Enteromorpha intestinalis*, a green seaweed species, against microorganisms isolated from fresh fruit juices. The isolates were characterized using 16S rRNA sequencing. Antimicrobial activity was assessed through disc diffusion and well diffusion assays against selected bacterial and fungal pathogens. Phytochemical analysis confirmed the presence of various bioactive compounds including alkaloids, flavonoids, phenols, tannins, steroids, and terpenoids. The methanolic extract exhibited moderate antifungal activity but lacked significant antibacterial properties. Thin Layer Chromatography (TLC) analysis revealed multiple bioactive compounds, and PCR was employed to verify molecular identity. The findings support the potential of *E. intestinalis* as a source of natural antifungal agents in food preservation and pharmaceutical applications.

INTRODUCTION

Natural products derived from marine algae are gaining recognition for their bioactive properties, especially in combating antibiotic-resistant pathogens. Seaweeds, including *Enteromorpha intestinalis*, are known to harbor antimicrobial constituents such as phenolics, alkaloids, flavonoids, and terpenoids (Cowan, 1999; Savoia, 2012). These compounds interfere with microbial growth and metabolism, making them candidates for natural therapeutics and preservatives.Fruit juices, although rich in vitamins, may harbor microbial contaminants due to exposure during processing. Targeting these contaminants with natural antimicrobial agents such as seaweed extracts offers a sustainable and health-conscious solution.This study focuses on evaluating the antimicrobial and phytochemical potential of methanolic extracts of *Enteromorpha intestinalis* against bacterial and fungal isolates from fresh juices, using standard biochemical and molecular methods.

2.LITERATURE REVIEW

1. Antimicrobial Activity of Enteromorpha intestinalis

Numerous studies demonstrate that methanolic extracts of *E. intestinalis* possess significant antimicrobial activity, particularly against Gram-negative bacteria and fungi.Erdoğan Eliuz et al. [1] reported that methanolic extracts of *Enteromorpha* incorporated in gelatin film inhibit bacterial growth on food surfaces.Swathi et al. [2], [13] and Pradhan et al. [4] observed moderate to strong antibacterial activity from *Enteromorpha* crude extracts.Alghazeer et al. [6] found flavonoid-rich extracts of *E. intestinalis* effective against multidrug-resistant strains.Al Mousawi et al. [7] demonstrated in vitro and in vivo efficacy against sarcoptic mange mites.Studies [3], [8], [31] confirmed methanolic extracts inhibited juice-derived microbial isolates.

2. Antifungal and Broad-Spectrum Activity

Watee et al. [5], [16] found *Ulva intestinalis* and *Enteromorpha* exhibited potent antimicrobial activity across multiple pathogens.Alghazeer et al. [6] and Al Mousawi et al. [7] highlighted broad-spectrum antimicrobial action including fungi and mites.Open-access studies [25], [18], [19] detailed inhibition of Gram-negative and fungal pathogens from clinical and food samples.

3. Phytochemical Composition and Bioactivity

Several papers focus on phytochemicals such as flavonoids, terpenoids, and phenolics, which are primarily responsible for antimicrobial action.Pradhan et al. [4] and Swathi et al. [2], [23] confirmed the presence of active secondary metabolites using phytochemical screening.“Phenolic compound derived…” [10] and [30] demonstrated that bioactive components correlate with observed antimicrobial efficacy.Bio-conferences and GC–MS-based studies [15], [30], [32] confirmed the richness of phytoconstituents like ulvans, alkaloids, and tannins.

4. Solvent Comparison and Extraction Techniques

The role of solvents in extracting bioactive compounds was examined in several comparative studies.Watee et al. [5], [16], [33] compared aqueous, ethanol, and methanol extracts and found methanol superior in extracting antibacterial compounds.Devati et al. [15] and [26] used GC–MS to identify compounds from different extraction protocols.Studies [24], [34] confirmed that methanolic extraction yields higher antimicrobial response than other solvents.

5. Food Preservation and Application in Packaging

The application of *E. intestinalis* extracts in food safety and preservation is highlighted.Erdoğan Eliuz et al. [1] and [29] applied methanolic extracts as part of edible coatings and films, reducing microbial contamination.Studies [9], [17], [28] supported the role of *Enteromorpha* in inhibiting spoilage organisms in food systems.Algae-based antimicrobial films evaluated in [32] showed significant preservation potential in juice and seafood.

6. Species Comparison and Geographic Variability

Variations in antimicrobial activity based on species and location were observed.[11], [12], [20], [21] compared *Ulva intestinalis*, *U. lactuca*, and *U. faciata*, highlighting species-specific bioactivity.Geographical comparison (e.g., Indian coasts, Caspian Sea) by [20], [35] revealed that habitat affects metabolite concentration and activity.

***7*. Antioxidant and Other Biofunctional Properties**

Besides antimicrobial action, *E. intestinalis* also exhibits antioxidant and anti-inflammatory activity.Pradhan et al. [4] confirmed anti-diabetic and anti-inflammatory properties.Studies [14], [22] evaluated anticancer potential of crude extracts.Research [21] and [27] also highlighted antioxidant enzyme boosting and metal chelation capacity.

3. MATERIALS AND METHODS

3.1 Collection and Preparation of Extract

Fresh samples of the green macroalga *Enteromorpha intestinalis* were collected from the intertidal zone of [Insert specific coastal location] under sterile conditions. The samples were thoroughly rinsed with sterile seawater followed by distilled water to remove epiphytes, sand particles, and salt residues. They were then shade-dried at room temperature for 5–7 days to preserve thermolabile bioactive compounds. Once dried, the material was ground into a fine powder using a sterile mechanical grinder and stored in airtight containers.

Extraction Protocol:

- **Solvent**: 90% methanol (analytical grade)
- **Ratio**: 1:10 w/v (10 g seaweed powder in 100 mL methanol)
- **Method**: Cold maceration for 72 hours with intermittent shaking
- The mixture was filtered through Whatman No. 1 filter paper
- The filtrate was concentrated using a rotary evaporator at 40–45°C under reduced pressure
- The crude extract was stored in airtight vials at 4°C for further use

3.2 Culture Medium

Bacterial Cultures

The bacterial isolates obtained from fresh juice samples were grown and maintained on Nutrient Agar (NA) medium at 37°C for 24 hours.

Step	Temperature	Time
Initial Denaturation	95°C	2 min
Denaturation	95°C	30 sec
Annealing	55°C	30 sec
Extension	72°C	2 min
Final Extension	72°C	10 min
Hold	4°C	∞

Table 1:PCR Conditions

3.6 Thin Layer Chromatography (TLC)

TLC was performed to detect the presence of bioactive compounds in the methanolic extract.

Procedure:

Fungal Cultures

Fungal strains were cultured on Potato Dextrose Agar (PDA). The pH of PDA was adjusted to 3.5 using 10% tartaric acid to selectively enhance fungal growth and suppress bacterial contamination.

3.3 Antimicrobial Assay

The disc diffusion method was employed to evaluate antimicrobial activity.

- **Extract concentrations tested**: 500 µg/disc, 1000 µg/disc, and 2000 µg/disc
- **Solvent control (negative control)**: Methanol alone
- **Positive controls**:
  - **Streptomycin (20 µg/disc)** for bacterial isolates
  - **Ketoconazole (20 µg/disc)** for fungal isolates

Procedure:

1. Sterile discs (6 mm) were impregnated with varying concentrations of the seaweed extract.
2. Each disc was air-dried and placed on Mueller-Hinton Agar (for bacteria) and PDA plates (for fungi) previously seeded with the test microorganisms.
3. Plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 48–72 hours (fungi).
4. Zones of inhibition were measured in millimeters (mm) using a digital caliper.

3.4 Phytochemical Screening

Aqueous extracts of *E. intestinalis* were subjected to qualitative phytochemical analysis to detect the presence of:

- **Alkaloids** (Mayer's & Wagner's test)
- **Saponins** (froth test)
- **Tannins** (ferric chloride test)
- **Flavonoids** (alkaline reagent test)
- **Phenols** (lead acetate test)
- **Steroids** (Salkowski's test)
- **Glycosides** (Keller-Killiani test)
- **Terpenoids** (Salkowski's test)
- **Quinones** (alkaline reagent test)
- **Proteins** (Biuret test)

3.5 PCR and 16S rRNA Sequencing

DNA Isolation and Amplification:

- Genomic DNA was extracted from bacterial isolates using the phenol-chloroform method.
- Amplification was performed using universal 16S rRNA primers:
  - Forward primer: 5'-AGAGTTTGATCCTGGCTCAG-3'
  - Reverse primer: 5'-TACGGTTACCTTGTTACGACTT-3'

1. **TLC Plates**: Pre-coated silica gel 60 F254
2. **Mobile Phase**: Chloroform:Methanol (90:10)
3. **Sample loading**: 10 µL of extract using micropipette

4. Plates were developed in TLC chamber until the solvent front reached ~80% of the plate

$$Rf = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

5. Visualization was done under UV light at 254 nm and 366 nm
6. Rf values were calculated using:

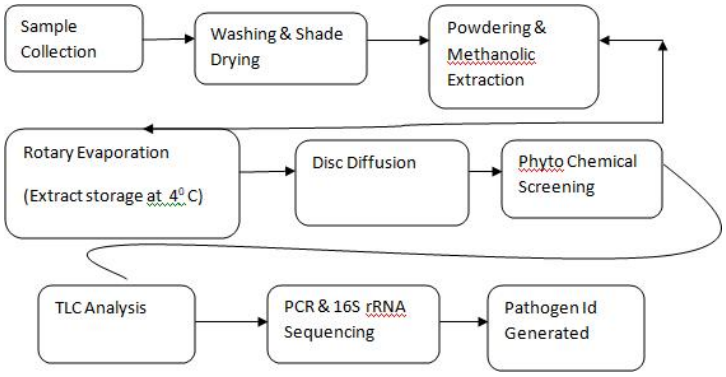

Figure no 1: Block Diagram of the Proposed Work

4. RESULTS AND DISCUSSION

4.1 Antimicrobial Activity

The extract exhibited no antibacterial activity, as no inhibition zones were observed at any concentration tested. However, moderate antifungal activity was observed against *Aspergillus fumigatus* at 1000 µg and 2000 µg concentrations with inhibition zones of 7 mm and 9 mm, respectively. Ketoconazole, used as the control, produced a 26 mm inhibition zone.

4.2 Phytochemical Analysis

Compound Group	Presence (+/-)
Alkaloids	+
Saponins	+
Tannins	+
Cardial glycosides	+
Flavonoids	+
Phenols	+
Steroids	+
Terpenoids	+
Quinones	+
Proteins	+

Table 2: Presence of Analysis

4.3 PCR Amplification

PCR confirmed the identity of the bacterial isolates via 16S rRNA sequencing. Amplification was successful, and standard cycling parameters were maintained:

Stage	Temperature	Time
Initial Denaturation	95°C	2 min
Denaturation	95°C	30 sec
Annealing	55°C	30 sec
Extension	72°C	2 min
Final Extension	72°C	10 min
Hold	4°C	∞

Table 3: Temperature Analysis with time

4.4 TLC Results

Five distinct spots were detected with Rf values: 0.26, 0.43, 0.60, 0.86, and 0.96. This indicates the presence of multiple phytoconstituents, supporting the results from phytochemical screening.

CONCLUSION

The methanolic extract of *Enteromorpha intestinalis* demonstrated moderate antifungal activity but lacked antibacterial properties in this study. The phytochemical constituents such as flavonoids, tannins, and phenols likely contributed to the observed effects. While the antimicrobial potential is limited, the extract's rich phytochemical profile warrants further exploration, especially for food preservation and antifungal formulations.Perform quantitative phytochemical analysis (e.g., HPLC, GC-MS) for compound profiling.Investigate synergistic effects with known antibiotics.Evaluate cytotoxicity and safety in model organisms.Formulate biodegradable antimicrobial coatings using seaweed extracts for food packaging.Conduct broader-spectrum microbial testing including Gram-positive and Gram-negative bacteria.

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