

ANTIMICROBIAL ACTIVITY OF *ENTEROMORPHA INTESTINALIS*

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ABSTRACT

The present study aimed to evaluating the in vitro antimicrobial activity of 90 % Methanolextractofseaweed *Enteromorpha Intestinalis* against isolated Microorganisms from fresh juice. The isolated microorganisms were identified by using 16s rRNA sequence. The potential use of Seaweed. Extract against isolated microorganisms from fresh juice. The phytochemical analysis of plant extract some commonly occurring seaweed. *Enteromorpha Intestinalis* There evaluated for Antimicrobial activity by well Diffusion assay was studied. Found to be drying process has eliminated the active principles in these seaweed. The extracts were tested for antimicrobial activity against a variety of bacteria and fungi. Traditional medicine makes use of a variety of plant extracts or bioactive ingredients. The presence of numerous phytochemicals such as alkaloids, saponins, tannins, cordial glycosides, flavonoids, phenols, steroids, terpenoids, quinones, and proteins has been confirmed by qualitative phytochemical analysis of this seaweed.

Keywords: *Enteromorpha Intestinalis*- Phytochemical; Antimicrobial; Thin Layer Chromatography

1. INTRODUCTION

Because of their affordability, reliability, availability, and low toxicity in therapeutic applications, medicinal plants have become popular and accepted for use in medical health care across the world. Plants were unquestionably the first material used in alternative medicine to cure a range of diseases (Amjad K et al., 2005). Medicinal herbs might be a source of previously unknown antibacterial chemicals. Herbal medications are utilised by 4 billion people (or 80% of the world's population) for some aspect of primary healthcare, according to the World Health Organization (WHO) (Fabricant DS and Farnsworth NR, 2001). Traditional treatments based on plant-based components are utilised by 80 percent of people in poor countries. In many nations, macroalgae is a significant coastal source for human consumption and the environment (Ratana-arporn and Chirapart, 2006). Since ancient times, it has been employed in numerous industries such as food, medicine, cosmetics, and agriculture due to its health advantages.

The relevance of algae in human nutrition stems from the fact that it contains all of the required elements in the right amounts for a balanced diet. Because of the rich protein, vitamins, and minerals in its structure, as well as the low fat content, algae is second only to fish in terms of health benefits. Algae's chemical structure is affected by seasonal variations in their life cycle (etingül, 2001). Macroalgae, which are good to human health, may be employed in a variety of industries. Many nations use macroalgae as a food source because of its nutritional value, however despite the fact that our country is surrounded by sea, has various inland waterways, and a diverse flora, macroalgae is not favoured as a food source. Because of their high protein, vitamin, and mineral content, green algae (Chlorophyta) are commonly employed in the food sector (Ova Kaykaç et al., 2008). The bulk of marine algae might be used as direct and indirect human food sources, as well as in new pharmaceutical and antibiotic companies (Oh et al., 2008; Rajasulochana et al., 2009). Marine species have a plethora of structurally novel and physiologically active chemicals (Ely et al., 2004 and Said et al. 2013). Human infections have received significant attention in terms of antiviral, antibacterial, and/or antifungal properties (Li et al., 2007; Rajasulochana et al., 2009).

El-Fatimi and Said (2007) describe an ideal antimicrobial agent as one that has selective toxicity, meaning that it solely harms the host (Brooks et al., 2007). (2011a). A high concentration of methanolic extract (100 m) of the green alga *Caulerpa prolifera* of Ghemini coast was used to heal nails and hair dermatophytes or Tinea (Kulkarni et al., 1993) *Alternaria brasica* (Kamble et al., 2012). A high concentration of methanolic extract (100 m) of the green alga *Caulerpa prolifera* of the Ghemini coast was used to create *Alternaria brasica* (Kamble et al., 2012). The present study aimed to analyse the anti microbial activity of the seaweed methanolic juice on different microbial organisms.

2. MATERIALS AND METHODS

The seaweed, *Enteromorpha Intestinalis* was collected from the seashore region at Rameshwaram (9.288°N 79.313°E), Tamilnadu, India. The material was collected and preserved in plastic bags before being delivered to the lab on ice. The sample was first completely rinsed with sea water to remove sand and any clinging substance, then extensively washed with fresh water to remove salts before being kept (30g) at -20° C until compound extraction.



Fig 1- *Enteromorpha intestinalis*

The seaweed sample was rinsed many times with distilled water before being washed again with 5% ethanol to remove any epiphytes or salts. The sample was allowed to dry naturally in the shade. They were crushed into a powder using an electrical mixer after drying. The powdered material was then kept in a dark location before being extracted. Methanol (90%) was used to extract the powdered seaweed sample. For 72 hours, aliquots of 25 g powdered seaweed sample were soaked in 250 ml of the solvent. The soaking samples were then homogenised with the solvent in an electric blender at ambient temperature, filtered, and concentrated under reduced pressure using a rotary evaporator before being stored at -20° C. Alkaloids, terpenoids, saponins, tannins, glycosides, flavonoids, Quinones, and ninhydrins were all tested for phytochemicals. The disc diffusion technique was used to test the antibacterial activity of plant extract. To establish the sensitivity of the microbiological species examined, the standard antibiotic Streptomycin (20g) was employed as a positive reference standard. The inoculation plates were then incubated for 24 hours at 37 degrees Celsius. The antibacterial activity of the clearzone surrounding the disc was measured and quantified in millimetres. GREENSMED Microbiology Laboratory provided the bacterial cultures utilised in this investigation. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* were among the bacteria used in this investigation. Nutrient agar media was used to cultivate all of the cultures. The antibacterial test was performed using the inoculum. The fungal cultures utilised in this work were from the CAS in Botany Laboratory's Culture Collection Center. *Aspergillus niger* and *Candida albicans* were the fungal cultures used in this investigation. Potato dextrose agar was used to grow all of the cultures (Media). The antifungal test was performed using the inoculum. Thin layer chromatography (TLC) was performed on seaweed extract using silica gel 60F254, which was cut into 5x3 cm (Merck) using a TLC cutter. After the elution, the plates were dried and viewed in a UV chamber before being sprayed with several spray reagents. The following formula was used to calculate Rf values: $Rf = \text{Distance travelled by the solute} / \text{Distance travelled by the solvent}$

3. Results and Discussion

The powdered material was kept in a dark area and extracted using a technique. Methanol was used to extract the powdered seaweed sample. For 72 hours, aliquots of 25 g powdered seaweed sample were soaked in 250 ml of the solvent. The soaking samples were then homogenised with the solvent in an electric blender at ambient temperature, filtered, and concentrated under reduced pressure using a rotary evaporator before being stored at -20° C. Figure 2 shows an example of this.



Fig2- *Enteromorpha intestinalis* extract

The present study of *Enteromorpha intestinalis* reveals the presence of qualitative phytochemical constituents such as

alkaloids, carbohydrate, glycosides, saponins, protein aminoacids, aminoacids, phenol, flavonoid, terpenoids, and steroids in various solvent extracts. Quantitative analysis reveals the presence of bioactive compounds such as phenols and flavonoids in various solvent extracts. Methanol extract has a strong activity in phytochemical analysis, as demonstrated in fig 3.

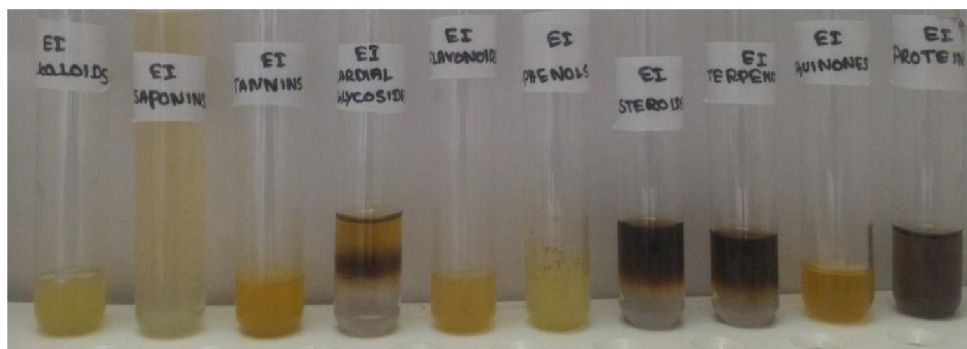


Figure 3- Phytochemical analysis of Enteromorpha intestinalis extract

In phytochemical analysis methanol extract shows high activity. As shown in table 1

Table 1- Phytochemical analysis of Enteromorpha intestinalis extract (+Present)

Phytochemicals	Enteromorpha intestinalis extract
Alkaloids	+
Saponins	+
Tannins	+
Cardiolglycosides	+
Flavonoids	+
Phenols	+
Steroids	+
Terpenoids	+
Quinone	+
Proteins	+

The antibiotic disc diffusion technique was used to assess the antibacterial activity of Enteromorpha intestinalis extracts (methanol) on nutritional agar medium. On microbial strains, the extracts of Enteromorpha intestinalis were tested for antibacterial activity. The greatest zone was recorded in methanolic extract in 0.086mm/g concentration against Bacillus subtilis using Enteromorpha intestinalis extracts. Table 2 shows the results.

Table 2 – Antibacterial Activity of Enteromorpha intestinalis

Sample	Zone of Inhibition (mm)
	<i>Bacillus subtilis</i>

	500 µg	1000 µg	2000 µg
EI	-	-	-
treptomyc in(20 µg)	21		

The antibiotic disc diffusion technique was used to assess the antibacterial activity of *Enteromorpha intestinalis* extracts (methanol) on nutritional agar medium. The maximum zone was reported in methanolic extract against *Bacillus subtilis* using *Enteromorpha intestinalis* extracts. Figure 4 illustrates this.

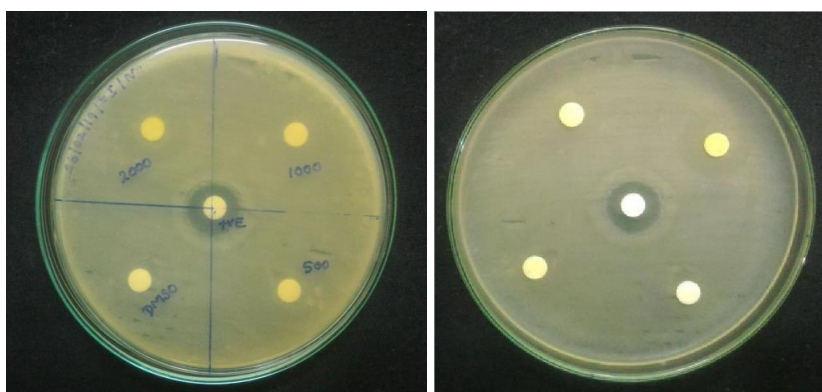


Fig 4– Antibacterial of disc diffusion method (*Bacillus subtilis*)

The antibiotic disc diffusion technique was used to assess the antifungal activity of *Enteromorpha intestinalis* extracts (methanol) on nutritional agar medium. On microbial strains, the extracts of *Enteromorpha intestinalis* were tested for antibacterial activity. The greatest zone was reported in methanolic extract in 26mm/500g concentration against *Aspergillus fumigatus* using *Enteromorpha intestinalis* extracts. Figure 5 shows an example of this.

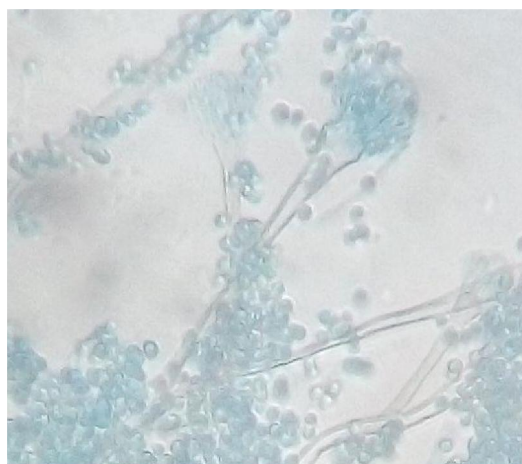


Fig 5- Using LPCB method on *Aspergillus fumigatus*

The antibiotic disc diffusion technique was used to assess the antifungal activity of *Enteromorpha* intestinalis extracts (methanol) on nutritional agar medium. On microbial strains, the extracts of *Enteromorpha* intestinalis were tested for antibacterial activity. *Enteromorpha* intestinalis extracts were reported to have the maximum zone of activity against *Aspergillus fumigatus* in a methanolic extract. Table 3 shows the results.

Table 3- Antifungal activity of *Enteromorpha* intestinalis

Sample	Zone of Inhibition (mm)		
	<i>Aspergillus fumigatus</i>		
	500µg	1000 µg	2000 µg
EI	-	7	9
Ketoconazole (20 µg)	26		

Antifungal activity of *Enteromorpha* intestinalis extracts (methanol) were determined by antibiotic disc diffusion method on nutrient agar medium. The *Enteromorpha* intestinalis extracts were tested for antibacterial activities on microbial strains. The *Enteromorpha* intestinalis extracts were found to the highest zone were observed in methanolic extract was against *Aspergillus fumigatus*. As shown in fig6.

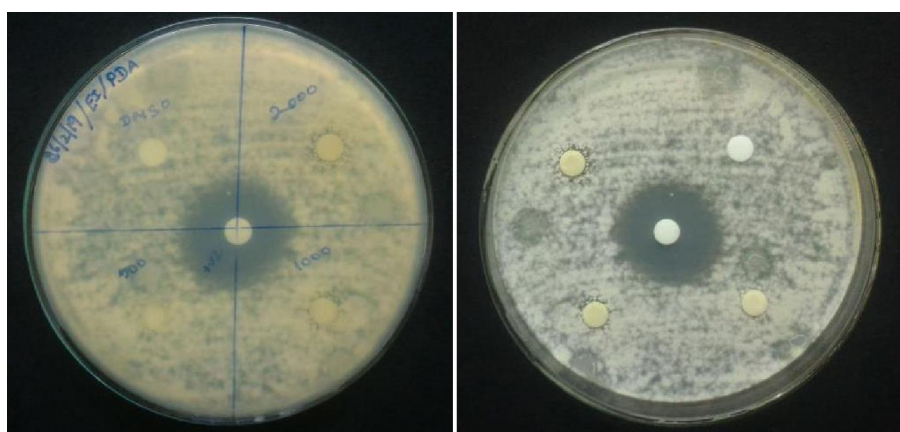


Fig 6– Antifungal on disc diffusion method (*Aspergillus fumigatus*)

In TLC, any substances that can be finely divided and formed into a uniform layer can be used. In the present study, TLC of *Enteromorpha* intestinalis in methanol extract were shown high activity. As shown in fig 7.



Figure 7- TLC OF ANALYSIS

Both organic and inorganic substances can be used to form a uniform layer for TLC. In the present study, TLC of *Enteromorpha intestinalis* methanol extract were shown high activity. As shown in table 4.

Table 4-TLC of *Enteromorpha intestinalis* extract

Extract	Solvent system	Number of spots	Rf value
Methanol	Chloroform:Methanol (90:10)	5	0.26; 0.43; 0.6; 0.86; 0.96

4. Conclusion:

When it comes to the antibacterial effect of fresh fruit juices, the study concludes that fresh juice has the greatest impact on suppressing microbe development, followed by fresh juices. As a result, drinking fruit juice on a daily basis can be a beneficial preventative strategy for maintaining dental hygiene and overall health. Isolation and further examination of the phytochemical will lead to the creation of many additional plant extracts for medicinal use in a variety of ailments.

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Ethical approval: The study was approved by the Institutional Ethics Committee

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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