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Evaluation of different seaweed extracts against *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

Seaweeds have gained significant attention in agriculture due to their diverse applications, particularly as a source of bioactive compounds with antimicrobial properties. Green, brown, and red algae are known to contain substances that exhibit antibacterial activity and inhibit the growth of pathogenic microbes. This study aimed to evaluate the efficacy of different seaweed extracts in suppressing Xanthomonas oryzae pv. oryzae, a major pathogen in rice. Among the tested extracts, Sargassum wightii at 20% concentration demonstrated the highest antibacterial activity, reducing bacterial colony formation to 1.8 x 10^5 CFU/mL. These findings suggest that seaweed extracts, particularly Sargassum wightii, could serve as a natural and effective alternative for managing bacterial diseases in crops.

Keywords: *seaweed, diseases, Sargassum wightii, rice cultivation, extracts*

Introduction

India leads the world in rice cultivation, with an area of 43 million hectares and an annual production of 87.80 million tons. To meet the national target of 140 million tons of rice production by 2025, an increase of 2 million tons per year is required [1]. However, achieving this goal is challenged by various diseases caused by fungal, bacterial, and viral pathogens. Among these, bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), has emerged as a serious threat to rice production in both tropical and temperate regions due to its high epidemic potential. Currently, BLB is managed through chemical control measures, such as the application of copper oxychloride and streptomycin sulfate. While effective, excessive use of these chemicals poses significant risks to the environment and public health [2]. This underscores the need for sustainable, eco-friendly alternatives that not only protect crops but also safeguard environmental and consumer well-being. Exploring biological solutions, such as seaweed-based extracts with antimicrobial properties, offers a promising approach for managing bacterial diseases while promoting healthier crop production.

Materials and Methods

Collection and preparation of seaweed extracts [3]

Seaweeds were collected from the Gulf of Mannar along the Mandapam coast, Tamil Nadu. The samples included various brown algae species, such as *Sargassum wightii, Sargassum ilicifolium, Turbinaria conoides*, and the green algae Ulva fasciata. These seaweeds were manually collected by hand picking. After collection, the seaweeds were thoroughly washed with seawater to remove any adhering epiphytes and extraneous materials. Subsequently, they were rinsed with fresh water 4-5 times to ensure cleanliness. The cleaned seaweeds were then dried in the shade to retain their bioactive properties.

For the extraction process, 100 grams of dried, powdered seaweed were mixed with distilled water at a 1:10 ratio and autoclaved at 150 psi for one hour. The extracts were immediately filtered through muslin cloth to remove solid particles.

The resulting seaweed extracts were measured, labeled, and stored in clean bottles, which were kept in a refrigerator to preserve their potency. These extracts were considered as 100% Seaweed Concentrate (SWC). Various concentrations of SWC were prepared by diluting with distilled water for use in the present study.

Poisoned Food Technique [4]

Experimental Procedure

A loopful of Xanthomonas oryzae pv. oryzae (Xoo) was inoculated into 90 mL of peptone sucrose broth and incubated in a rotary shaker at 150 rpm for 12 hours at room temperature (28±2°C). Following incubation, 10 mL of various concentrations (5%, 10%, 15%, and 20%) of seaweed extracts were added separately to 90 mL of the broth, achieving a 1:10 dilution. The cultures were then incubated for an additional 12 hours under the same conditions (150 rpm, 28±2°C).

After incubation, 1 mL of each mixture was subjected to serial dilution to achieve suspensions with concentrations of 10^{-5}, 10^{-6}, and 10^{-7}. From each dilution, 1 mL was combined with 20 mL of peptone sucrose agar medium and poured into sterile Petri dishes. As control treatments, plates containing media amended with either sterile distilled water or streptomycin (100 ppm) were prepared. All plates were incubated at room temperature (28±2°C), and the number of bacterial colonies was recorded after 24 hours. Each treatment was replicated three times to ensure accuracy and reliability of the results.

Agar well method [5]

The antibacterial activity of the seaweed extracts against Xanthomonas oryzae pv. oryzae (Xoo) was assessed using the agar well diffusion method. A bacterial inoculum of Xoo (1x10^6 CFU/mL) was uniformly spread onto nutrient agar plates. Wells of 5 mm diameter were created on the agar surface using a sterile cork borer. Seaweed extracts at varying concentrations (2.5%, 5%, 10%, 15%, and 20%) were carefully dispensed into the wells using a sterile syringe. Streptomycin (100 ppm) served as a positive control for comparison.

The inoculated plates were incubated at 28±2°C for 48 hours.

Each treatment was replicated three times. Following incubation, the plates were observed for the formation of inhibition zones around the wells, indicating antibacterial activity. The diameter of the inhibition zones, including the well diameter, was measured in millimeters to quantify the antibacterial efficacy of the seaweed extracts.

Result and Discussion

Evaluation of different seaweed extracts against Xoo 3 under *in vitro* condition (Poison food technique)

Four different seaweed extracts were evaluated at various concentrations against *Xanthomonas oryzae* pv. *oryzae* (Xoo), and the findings are summarized in Table 1. Among the extracts, Sargassum wightii at a 20% concentration exhibited the highest antibacterial activity, resulting in the lowest colony count (1.8 x 10^5 CFU/mL). This was closely followed by *Sargassum ilicifolium* at the same concentration. Lower concentrations of seaweed extracts in the growth medium did not demonstrate any significant inhibitory effect on bacterial growth, while increasing the concentration beyond 20% did not further enhance the antibacterial activity.

The standard antibiotic, streptomycin (100 ppm), served as a positive control and resulted in complete inhibition of *Xoo* growth, highlighting its superior effectiveness in comparison to the seaweed extracts. However, the promising results from Sargassum species suggest that they could serve as eco-friendly alternatives for managing bacterial pathogens in agriculture, reducing the reliance on chemical treatments.

Evaluation of different seaweed extracts against Xoo 3 under *in vitro* condition (Agar well method)

The present study evaluated the antibacterial activity of different seaweed extracts against Xanthomonas oryzae pv. *oryzae* (Xoo), and the results are detailed in Table 2. The zone of inhibition ranged from 9.5 mm to 17.6 mm across the various extracts and concentrations tested. *Sargassum wightii* at a 20% concentration demonstrated the strongest antibacterial activity, with a maximum inhibition zone of 17.6 mm, followed by *Sargassum ilicifolium* at the same concentration, which recorded an inhibition zone of 16.1 mm. In contrast, *Turbinaria conoides* was the least effective, exhibiting a zone of inhibition of only 9.5 mm.

These findings are consistent with the growing body of research highlighting the antimicrobial potential of seaweed-derived compounds. For example, the marine environment is known to harbor a wealth of bioactive compounds with potential applications in combating infectious diseases and parasites [6-7], identified 40 volatile compounds, including n-docosane, n-eicosane, and tetratriacontane, from the red seaweed *Jania rubens*, which showed potent antimicrobial activity. Similarly, methanol and chloroform extracts from *Sargassum ilicifolium* exhibited inhibition zones of 8 mm and 10 mm, respectively, against *Fusarium oxysporum* at concentrations of 4 mg/disc and 6 mg/disc. *Sargassum ilicifolium* was also effective against Macrophomina phaseolina, producing an inhibition zone of 10 mm [8]. Further supporting these findings, *Gracilaria chilensis* extracts have been shown to reduce the growth of *Phytophthora cinnamomi* under in vitro conditions [3]. The antibacterial compounds present in *Sargassum wightii* likely played a key role in suppressing the growth of *Xoo*, as evidenced by the significant inhibition zones observed in this study.

Table 1. Evaluation of different seaweed extract against Xoo3 under in vitro condition (Poison food technique)

S.No	Treatment sources	Number of colonies				
		2.5%	5%	10%	15%	20%
1.	*Sargassum ilicifolium*	7.4	5.5	3.2	3.1	3.0
2.	*Sargassum wightii*	6.5	5.0	2.0	1.8	1.8
3.	*Ulva faciata*	8.1	5.6	4.4	4.2	4.2
4.	*Turbinariaconoides*	10.2	6.7	5.6	5.5	5.3
5.	Streptomycin (100ppm)	0.0				
6.	Control	32.21				

Table 2. Evaluation of different seaweed extract against Xoo 3 under in vitro condition (Agar well method)

S.No	Treatment sources	Inhibition zone (mm)				
		2.5%	5%	10%	15%	20%
1.	*Sargassum ilicifolium*	7.2	10.5	15.7	15.9	16.1
2.	*Sargassum wightii*	9.8	12.9	17.5	17.6	17.6
3.	*Ulva faciata*	6.3	7.8	11.7	11.9	12.1
4.	*Turbinariaconoides*	5.5	6.8	8.3	8.3	9.5
5.	Streptomycin (100ppm)	11.2				
6.	Control	0.0				

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