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ORIGINAL ARTICLE

**Antagonistic Activities of Seagrass-Associated Epiphytic and Endophytic Bacteria against Multidrug-Resistant Organisms and Clinical Pathogens**

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ABSTRACT

Seagrass-associated bacteria are potential sources of novel antimicrobial compounds. This study explored epiphytic and endophytic bacteria from *Cymodocea serrulata*, *Halodule uninervis*, and *Syringodium isoetifolium* for their activity against multidrug-resistant organisms (MDROs) and clinical pathogens. Thirty-three isolates were obtained, mainly Gram-negative rods. An epiphytic strain from *Halodule uninervis*, showed highest inhibition (20 mm) against *Staphylococcus aureus* and significant antioxidant activity. MIC values ranged from 46.875–93.75 µg/mL. Bioassay-guided purification identified heptadecanoic acid as the major metabolite. SEM analysis confirmed cell wall damage in pathogens. Seagrass-associated bacteria, especially Epiphytic bacteria isolated from *Halodule uninervis*, hold promise as sources of antibacterial and antioxidant agents.

**Keywords:** Seagrass, Epiphytic bacteria, Antimicrobial, Antioxidant, Multidrug resistance

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INTRODUCTION

Antimicrobial resistance is a growing global threat, responsible for nearly 5 million deaths in 2019 [1]. Widespread misuse of antibiotics has accelerated the emergence of multidrug-resistant organisms (MDROs) such as MRSA, VRE, and carbapenem-resistant *Enterobacteriaceae*. Alternative resources are needed to combat this crisis. Marine ecosystems, particularly seagrasses, harbor diverse microorganisms capable of producing bioactive metabolites [2–4]. These microbial symbionts have been reported to possess antibacterial, antioxidant, and antifungal activities [4,5]. However, studies on seagrass-associated bacteria targeting MDROs remain limited. This research aims to isolate and characterize epiphytic and endophytic bacteria from selected seagrasses and evaluate their antagonistic activity.

MATERIAL AND METHODS

**Study Area and Sample Collection**

Healthy seagrasses were collected aseptically from the Mandapam coast, Tamil Nadu, India [6]. The site is characterized by shallow waters with sandy substrates and moderate salinity, providing a natural habitat for dense seagrass meadows. Samples were collected during low tide in sterile polythene bags and immediately transported to the laboratory on ice to minimize microbial contamination and degradation. Species included *Cymodocea serrulata*, *Halodule uninervis*, and *Syringodium isoetifolium*, identified according to standard taxonomic keys [6].

### **Isolation of Epiphytic and Endophytic Bacteria**

Epiphytic bacteria were obtained by gently scraping the leaf and rhizome surfaces using sterile scalpels [7]. For endophytes, plant tissues were washed under running seawater, surface-sterilized with 70% ethanol for 1 min, and rinsed three times in sterile seawater to remove external microbes. Sterilized tissues were aseptically cut into small sections, macerated, and streaked on Zobell Marine Agar. Plates were incubated at  $28 \pm 2$  °C for 48–72 h, and morphologically distinct colonies were sub-cultured for pure isolates. Bacterial identity was confirmed by Gram staining and basic biochemical characterization.

### **Preparation of Bacterial Extracts**

Purified bacterial isolates were grown in 250 mL Erlenmeyer flasks containing 100 mL nutrient broth. Cultures were incubated at 30 °C on a rotary shaker (150 rpm) for 3 days. Biomass and culture filtrates were extracted three times with equal volumes of ethyl acetate. The organic phase was collected, concentrated under reduced pressure using a rotary evaporator, and stored at 4 °C until further use.

### **Antibacterial Assays**

Antibacterial activity was evaluated by agar well diffusion [8]. Pathogenic test organisms included *Staphylococcus aureus*, MRSA, ESBL-producing *Escherichia coli*, and multidrug-resistant *Pseudomonas aeruginosa*. Standardized inoculum (0.5 McFarland,  $\sim 1 \times 10^8$  CFU/mL) were spread on Mueller–Hinton agar plates. Wells of 7 mm diameter were bored and filled with 100  $\mu$ L of extract. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured in millimeters. Ciprofloxacin (10  $\mu$ g/mL) served as the positive control, while ethyl acetate served as the negative control.

### **MIC and MBC Determination**

MIC values were determined by broth microdilution according to CLSI guidelines [9]. Serial two-fold dilutions of extracts (5–200  $\mu$ g/mL) were prepared in 96-well plates containing Mueller–Hinton broth inoculated with test pathogens. Wells were incubated at 37 °C for 24 h, and MIC was recorded as the lowest concentration inhibiting visible growth. MBC was determined by subculturing 10  $\mu$ L from wells without visible growth onto fresh agar plates and incubating for 24 h. The lowest concentration yielding no growth was recorded as the MBC.

### **Antioxidant Activity**

The antioxidant potential of extracts was assessed using multiple assays: ABTS radical cation decolorization [10], DPPH radical scavenging, phosphomolybdenum reduction, and ferric-reducing antioxidant power (FRAP). Each assay was performed in triplicate, and results were expressed as percentage inhibition or absorbance values. Ascorbic acid was used as a standard antioxidant for comparison.

### **Purification and Identification of Bioactive Compounds**

Active extracts were subjected to silica gel column chromatography using gradient elution with hexane and ethyl acetate. Fractions were analyzed by thin-layer chromatography (TLC), and bioactive spots were visualized under UV light and iodine vapor. The most active fraction was further analyzed by gas chromatography–mass spectrometry (GC–MS) and structural elucidation was performed using nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FT–IR) [11].

### **Scanning Electron Microscopy (SEM)**

To examine bacterial ultrastructural changes, pathogens treated with the bioactive fraction were fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, and dehydrated through a graded ethanol series. Samples were sputter-coated with gold and observed under a scanning electron microscope at an accelerating voltage of 15 kV. Untreated cells were used as controls to compare membrane integrity and cellular morphology.

## **RESULTS AND DISCUSSION**

Identification of seagrasses confirmed the presence of *Cymodocea serrulata*, *Halodule uninervis*, and *Syringodium isoetifolium*. Thirty-three bacterial strains were recovered (Table 1), predominantly Gram-negative rods, in line with earlier studies [4].

Antibacterial activity: Epiphytic bacteria from *H. uninervis* produced inhibition zones up to 20 mm against *Staphylococcus aureus* (MRSA), *E. coli* (ESBL), and *Pseudomonas aeruginosa* (MDR) (Fig. 1), demonstrating strong antimicrobial potential [2,3].

MIC and MBC: MIC values ranged 46.875–93.75  $\mu$ g/mL, while MBC values were 23.875–46.875  $\mu$ g/mL (Fig 2), confirming bactericidal effects.

Antioxidant properties: EPH7 showed dose-dependent ABTS radical scavenging (19.9 % at 80  $\mu$ g/mL) and high DPPH activity (Table 2 & Fig. 3), suggesting a dual benefit as an antioxidant.

Purification and characterization: Bioassay-guided fractionation identified the 10 % ethyl acetate fraction as most active. TLC revealed a principal spot (Rf 0.62) GC–MS and NMR identified heptadecanoic acid as the main bioactive metabolite, supporting its membrane-disruptive mechanism [11].

SEM observations: Treated pathogens exhibited cell wall rupture and cytoplasmic leakage (Fig. 4), contrasting with smooth, intact control cells.

**Table 1: Epiphytic and Endophytic bacterial load in sea grass**

Organism	Epiphytes	Endophytes
<i>Cymodaceae serrulata</i>	5	7
<i>Halodule uninervis</i>	8	5
<i>Syringodium isotefolium</i>	4	4

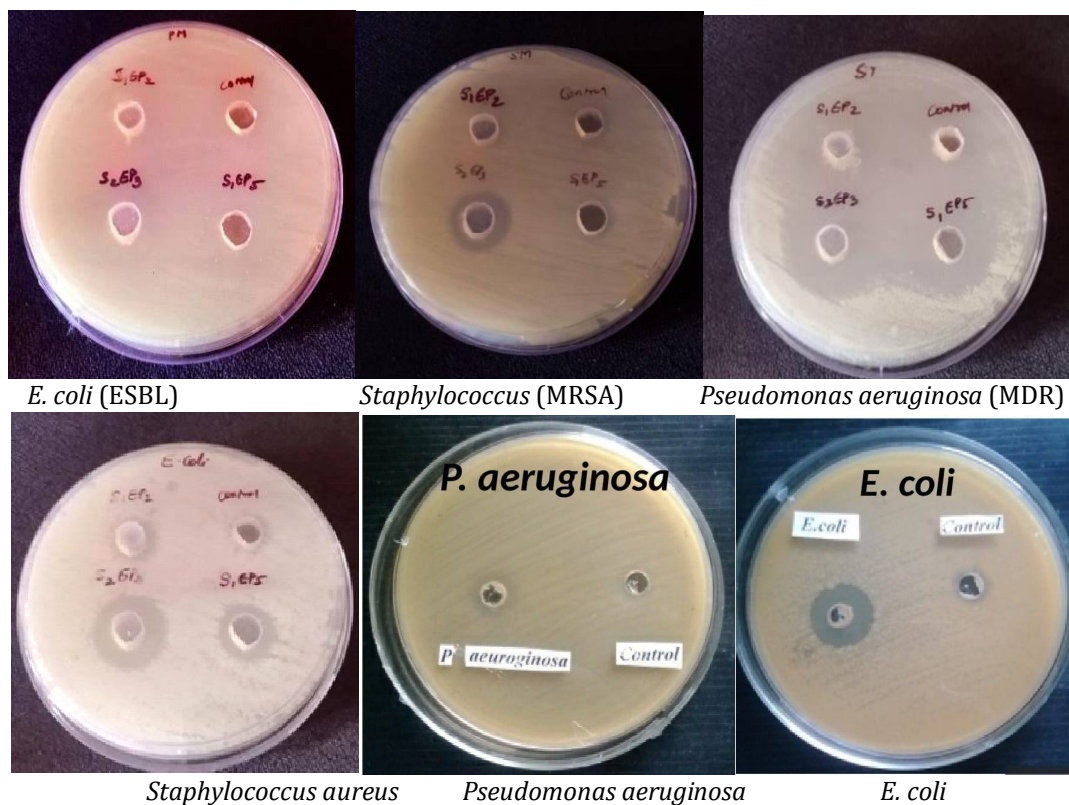
**Table 2. Evaluating the Antioxidant Activity of Epiphytic Bacterial Extract**

**Table 2 a: ABTS assay**

Concentration of the extract $\mu\text{g/mL}$	Sample	Scavenging %
100	0.594	18.5
80	0.587	19.9
60	0.688	2.32
40	0.698	0.86
20	0.698	0.86

**Table 2 b: DPPH Assay**

Concentration of the extract $\mu\text{g/mL}$	sample	Scavenging %
100	0.098	86.8
80	0.134	82
60	0.457	38.9
40	0.498	33.5
20	0.513	31.4



**Figure. 1: Antibacterial activity of epiphytic bacteria against MDR and clinical pathogens.**

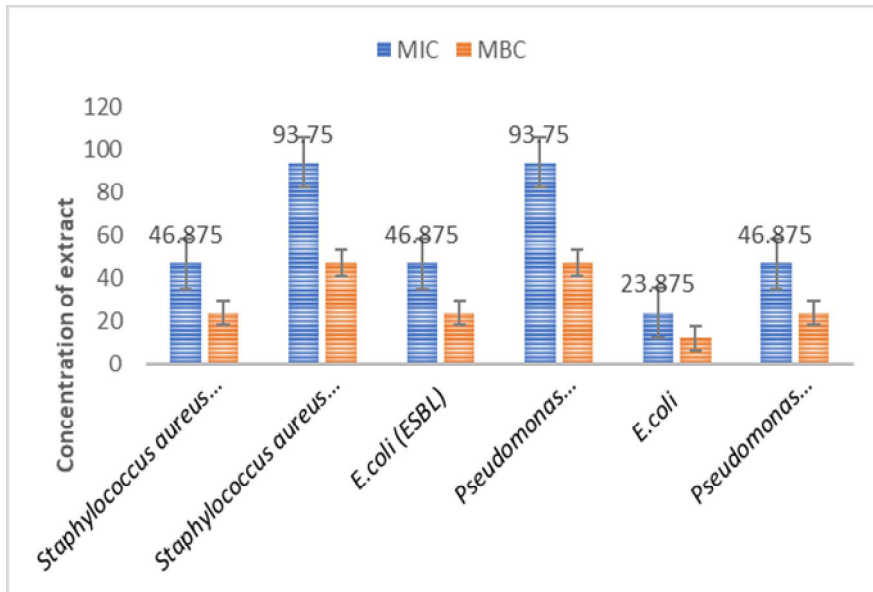


Figure.2. Minimum inhibitory concentration and Minimum bactericidal concentration of epiphytic bacteria associated with seagrass *Halodule uninervis*

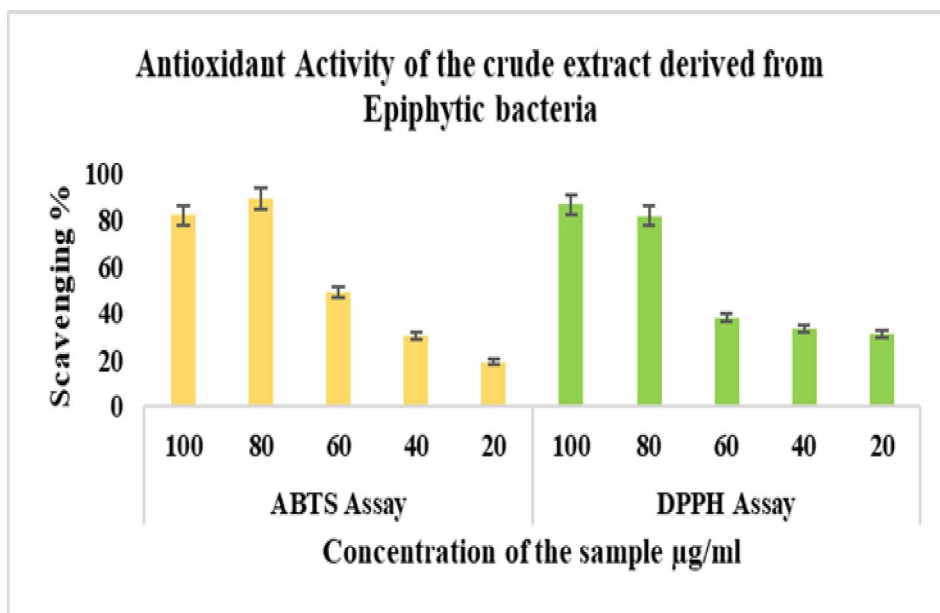
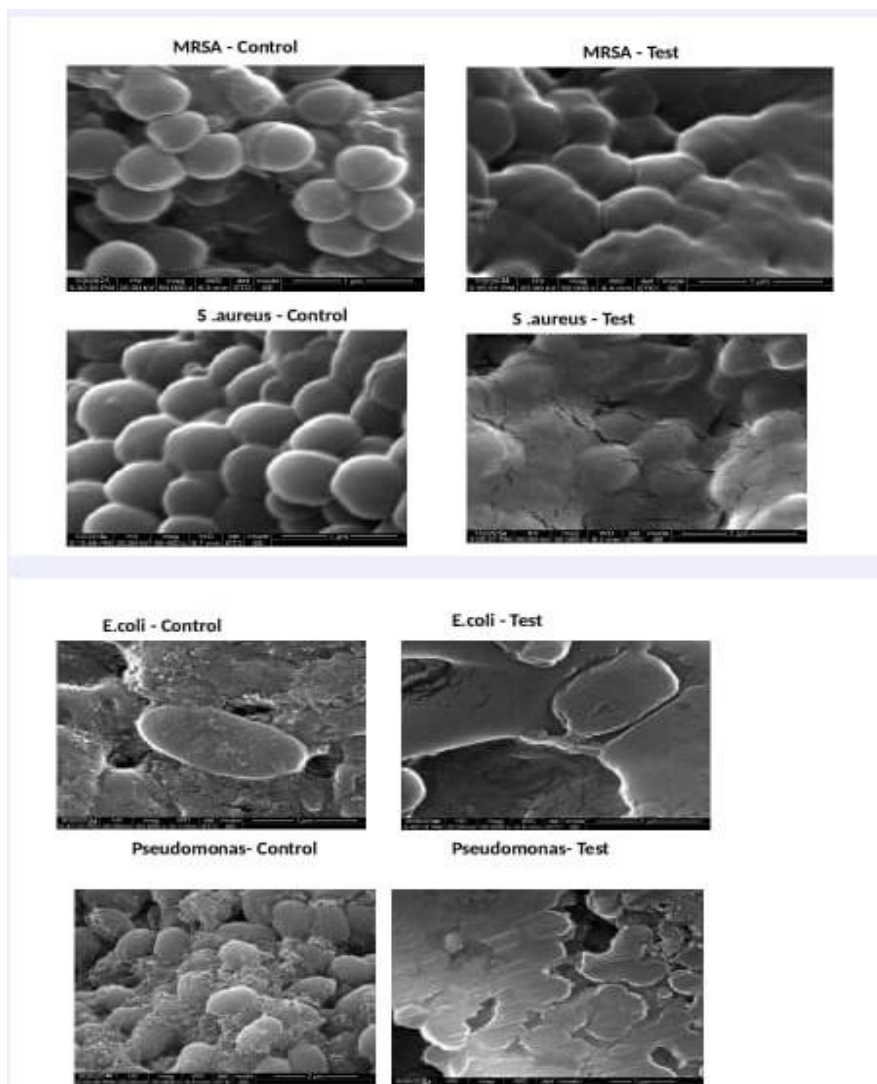


Figure. 3. Antioxidant activity of the crude extract derived from epiphytic bacteria



**Figure 4 : SEM analysis:** Treated pathogens exhibited cell wall rupture and cytoplasmic leakage, contrasting with smooth, intact control cells.

## CONCLUSION

Epiphytic bacteria from *Halodule uninervis*, produce heptadecanoic acid with potent antibacterial and antioxidant effects. Seagrass microbiota are promising candidates for developing new drugs against multidrug resistance.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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