**RESEARCH**
O&G Forum 2024; 34 – 2s: 445 - 448

# EVALUATION OF SEA GRASS METABOLITES ON ADHESION AND BIOFILM FORMATION OF CLINICAL PATHOGEN ESCHERICHIA COLI

**Pranam Sinha[1], Abirami Arthanari[2], Kamala[3]**
[1]Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Science (SIMATS)
Saveetha University, Chennai, India
[2]Senior Lecturer
Department of Forensic Odontology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Science (SIMATS)
Saveetha University,
Chennai - 600077, India
Mail ID: abiramia.sdc@saveetha.com
[3]Senior lecturer,
Department of Physiology,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical sciences (SIMATS),
Saveetha University,
Chennai – 600077
Tamil Nadu, India
Email ID: kamalak.sdc@saveetha.com.

**Corresponding author**: Dr. Abirami Arthanari
Senior Lecturer
Department of Forensic Odontology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Science (SIMATS)
Saveetha University,
Chennai - 600077, India
Mail ID: abiramia.sdc@saveetha.com

**Abstract**

***Introduction:*** *E.coli* is a gram negative, straight rod measuring 1-3µm x 0.4 - 0.7µm, arranged singly or in pairs. It is motile by peritrichous flagella, though some strains may be non-motile. Capsules and fimbriae are found in some virulent strains. A complex structure known as biofilm is made up of related microbial cells that are bonded to surfaces and contained in a matrix of polysaccharides.

***Aim:*** To evaluate *Halophila ovalis* seagrass metabolites on adhesion and the biofilm formation for a clinical pathogen *Escherichia coli.*

***Materials and methods:*** Seagrass sample collection was shadow dried to achieve a powdered mixture which was mixed with 100mL of methanol to create an aqueous solution. The said extract was filtered with Whatmans paper and used for further anti-microbial assays

***Results:*** The untreated biofilm of *E.coli* was treated with seagrass for a difference of 24 hours for 3 days and all the cells were found to be dead, confocal imaging showed dead cells after treatment.

***Discussions:*** Similar research by Azizi et al., utilising the brown seaweed *Sargassum muticum* revealed the importance of bio active components like amino, sulphate, carboxyl and hydroxyl groups in biosynthesis process.

***Conclusion:*** The population of microbes was reduced drastically, untreated deep orange became yellowish in colour. After 48 hours, there was destruction of dead cells. In confocal analysis, using the red filter, all cells were deemed dead.

***Keywords:*** *Escherichia coli, Halophila ovalis,* seagrass metabolites, biofilm formation, confocal analysis

## INTRODUCTION:

This research topic explores the potential impact of seagrass-derived compounds on the behaviour of the clinical pathogen *Escherichia coli*. Seagrasses, vital components of coastal ecosystems, possess a diverse array of metabolites with various ecological functions [1]. This study aims to investigate whether

these metabolites have the ability to influence the adhesion and biofilm formation of *E. coli*, a common bacterium responsible for a range of infections in humans.

Biofilm formation is a crucial virulence factor for *E. coli*, as it enables the bacteria to adhere to surfaces and form robust communities protected by a slimy matrix [2]. This matrix not only shields bacteria from the host immune system but also contributes to antibiotic resistance, making biofilm-associated infections challenging to treat [3][4]. By examining how seagrass metabolites impact adhesion and biofilm formation, this research could provide valuable insights into potential novel strategies to combat *E. coli* infections. In both natural and artificial settings, the capacity of microbes to colonise surfaces and evolve into highly structured colonies contained in self-produced polymeric matrices is the dominating growth mode [5][6][7] . This sort of lifestyle is known as biofilm, and it is distinguished by changes in microbial characteristics such as growth rates and gene transcription. Biofilms are extremely important for public health since they constitute 65-80% of microbial infections being addressed by physicians in the industrialised world [8][9]. The inclusion of indwelling medical devices amplifies the risk of biofilm development and subsequent infection even more. The bacteria *E. coli* and the polymorphic fungus *Candida albicans* are the most common causes of bloodstream infections and the most common microbes identified from contaminated medical equipment [10]. These biofilms, like any other biofilm, are significantly less susceptible to antimicrobial drugs and resistant to host immune clearance, complicating clinical infection management. Furthermore, lead compounds are in low supply in the antibacterial arena, and rising consumer aversion to synthetic treatments has prompted a hunt for more natural alternatives [11].

The study employs a combination of microbiological, biochemical, and molecular techniques. Seagrass metabolites are extracted, purified, and then tested for their effects on bacterial attachment and biofilm development. These experiments will likely involve observing bacterial growth on different surfaces, assessing the integrity of biofilms, and measuring the expression of key genes involved in adhesion and biofilm formation [12][13]. The antibacterial, antifungal, antialgal, antioxidant, anti-inflammatory, insecticidal, antimalarial, and vasoprotective effects of seagrass extracts have also been described [14][15][16]. A viable foundation for researching new antibiofilm actions at non-lethal doses is provided by the well-described characteristics of seagrass extracts.

## MATERIALS AND METHODS :

**Microorganism and culture:** The clinical isolates were obtained from Saveetha medical college and stored in airtight storage containers with proper label which has name of isolate, date of storage, name of culture medium and details of preservation solution. All microbes were identified based on Bergey's manual of determinative bacteriology (1923).

**Chemicals:** Acridine orange, propidium iodide- Dye Shiga toxin producing isolates were grown on chromogenic ESBL agar medium then enterobacteria enrichment broth has been used for the formation. E.coli was grown on Mannitol salt agar. Biofilm on coverslip has been formed on Mannitol salt broth. Luria-Bertani agar medium has been used for the growth of E. coli, Biofilm on coverslip has been formed on Luria-Bertani broth.

**Methods :** Cell viability has been analysed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay by the method of Peter and Toshima, 2023. (Peter, L. and Z.P.Toshima, 2023. Optimization of. Cell viability assay for drug sensitivity screens. Methods Mol.Biol., 2644:287-302)

## RESULTS:

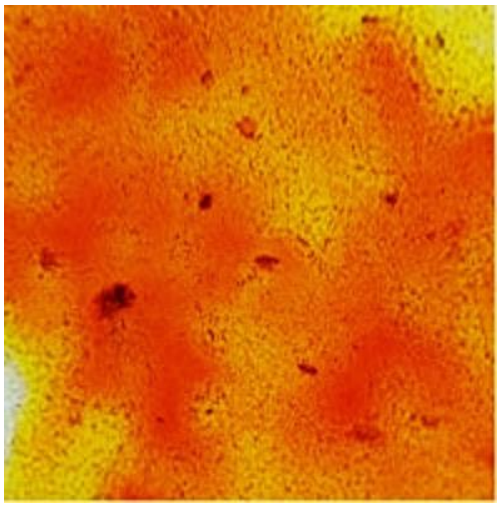

**Fig 1.1** The above image shows the untreated biofilm of *Eischeria coli*

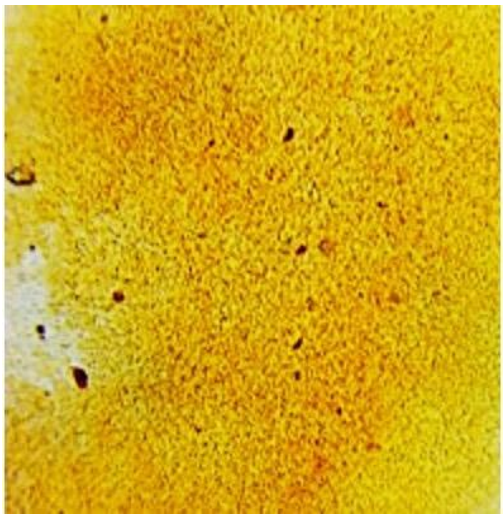

**Fig 1.2** The above image shows treated slide with seagrass after 24 hours

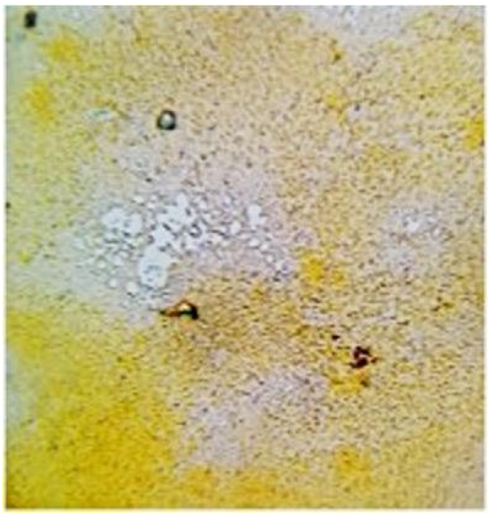

**Fig 1.3** The above image shows treated slide with seagrass after 48 hours

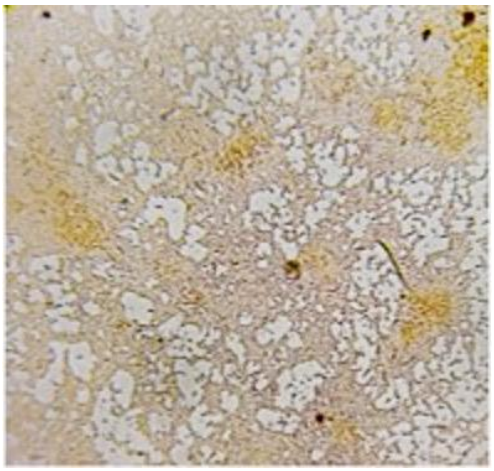

**Fig 1.4** The above image shows treated slide with seagrass after 72 hours

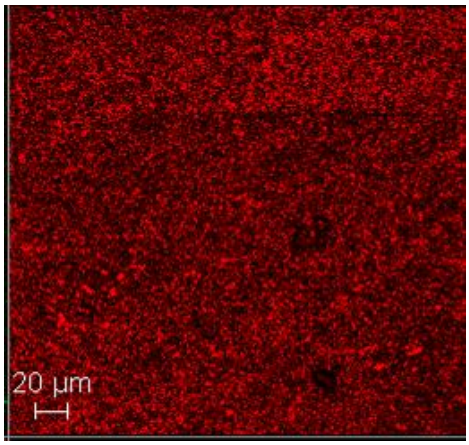


**Fig 1.5** Confocal Analysis of dead cells after treatment

| Identification of Pathogen | |
|---|---|
| **Gram stain** | - |
| **Shape** | Rod |
| **Motility** | + |
| **Indole** | + |
| **MR** | + |
| **VP** | + |
| **Citrate** | -- |
| **TSI** | -- |
| **Oxidase** | -- |
| **Catalase** | + |
| **Urease** | -- |
| **Lactose** | + |
| **Maltose** | + |
| **Sucrose** | ± |
| **Xylose** | ± |
| **Starch** | + |
| **Inosital** | -- |
| **Genus** | ***Escherichia*** |

**Table 1.1** The above table shows the identification of *E.coli.* For the present strain, the gram stain was negative, rod shaped, with motility, indole, MR, VP as positive tests and inositol as a negative test. Urease, oxidase, citrate and TSI were not found in the *E.coli* strain tested. Sucrose and xylose were a positive fail test.

**DISCUSSION:**

The investigation into the effects of seagrass metabolites on the adhesion and biofilm formation of the clinical pathogen Escherichia coli holds substantial promise for both medical and ecological domains. The study's findings have the potential to introduce groundbreaking strategies in the battle against antibiotic-resistant infections. By demonstrating the capacity of seagrass-derived compounds to hinder biofilm formation, the research paves the way for the development of innovative antimicrobial agents that can disrupt the protective shields of pathogenic bacteria. Moreover, this research underscores the intricate interplay between natural ecosystems and human health. The potential applications of seagrass metabolites not only offer therapeutic possibilities but also emphasise the value of biodiversity conservation. This study's interdisciplinary nature, bridging marine ecology and medical microbiology, exemplifies the potential for collaborative efforts to yield impactful solutions to complex challenges.

The bioactive characteristics of the seagrass species used for this study are well known and have been extensively studied by various authors. However, until recently, the majority of emphasis has been focused on the antibacterial activity of seagrass extracts, which were studied using disk diffusion tests not in their capacity as biofilm-forming microbes but in their planktonic stage [17][18]. Staphylococcus aureus, Staphylococcus epidermidis, and Candida albicans were tested using lethal concentrations to determine the antimicrobial properties of Zostera marina methanol extract and its organic solvent fractions. E. acoroides and other seagrass and seaweed species were tested for their antibacterial properties against various aquaculture pathogens [19][20].

As the global healthcare community grapples with the mounting threat of antibiotic resistance, the insights garnered from this research may inspire further investigations into other natural sources for novel antimicrobial compounds. Ultimately, the evaluation of seagrass metabolites and their impact on E. coli biofilms signifies a significant stride towards reshaping infection management strategies and cultivating a deeper appreciation for the inherent relationship between human well-being and the environment.

**CONCLUSION:**

The microbial population underwent a dramatic decline, causing the untreated deep orange substance to shift in color towards a yellowish tone. After 48 hours, the remnants of dead cells started to disintegrate. In a meticulous confocal analysis using a red filter, every cell was unequivocally classified as deceased. This transformation highlights the profound impact of the reduction in microbial numbers on the substance's appearance and composition. The shift from deep orange to yellow indicates the alteration of chemical processes, while the disappearance of live cells, as detected through the red filter, underscores the effectiveness of the treatment in eliminating the microbial population and its potential implications for various applications.

**SCOPE OF FUTURE RESEARCH :**

The clinical significance of the research lies in its potential to address the challenges posed by biofilm-associated E. coli infections. The findings could revolutionise infection management strategies, enhance the efficacy of treatments, and

contribute to the global effort to combat antibiotic resistance. Additionally, this research underscores the importance of exploring nature's resources for innovative solutions to medical problems, while also emphasising the interconnectedness of human health and the environment.

**CONFLICTS OF INTEREST :**
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**ACKNOWLEDGMENT :**
The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing research laboratory facilities and the required equipment to carry out the study successfully.

**AUTHOR CONTRIBUTION :**
**Pranam Sinha :** Literature search, Data collection analysis, Original manuscript draft
**Dr. Abirami Arthanari :** Data verification, Manuscript verification, Conceptualisation, Project administration
**Dr. Kamala :** Data collection analysis, Data verification, Conception or design of the work, Supervision of the findings

**REFERENCES :**
1. *Rodil IF, Lohrer AM, Attard KM, Thrush SF, Norkko A. Positive contribution of macrofaunal biodiversity to secondary production and seagrass carbon metabolism. Ecology. 2022 Apr;103(4):e3648.*
2. *Maunders E, Welch M. Matrix exopolysaccharides; the sticky side of biofilm formation. FEMS microbiology letters. 2017 Jul ;364(13):fnx120.*
3. *Kumar D, Kumar A. Cellular Attributes of Candida albicans Biofilm-Associated in Resistance Against Multidrug and Host Immune System. Microbial Drug Resistance. 2023 Jul 10.*
4. *Akanda ZZ, Taha M, Abdelbary H. Current review—the rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. Journal of Orthopaedic Research®. 2018 Apr;36(4):1051-60.*
5. *Costerton JW. Introduction to biofilm. Int J Antimicrob Agents. 1999;11:217-21.*
6. *Hall-Stoodley L, Costerton WJ, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2:95-108.*
7. *Rayner J, Veeh R, Flood J. Prevalence of microbial biofilms on selected fresh produce and household surfaces. Int J Food Microbiol. 2004;95:29-39.*
8. *Villa F, Villa S, Gelain A, Cappitelli F. Sub-lethal activity of small molecules from natural sources and their synthetic derivatives against biofilm forming nosocomial pathogens. Curr Top Med Chem. 2013;13(24):3184-204.*
9. *Macià MD, Rojo-Molinero E, Oliver A. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin Microbiol Infect. 2014;20(10):981-90.*
10. *Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk. tolerance and control J Med Microbiol 2015;64(Pt 4):323-34. 7.*
11. *Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012:4:165v113.*
12. *Dang H, Lovell CR. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. Applied and environmental microbiology. 2000 Feb 1;66(2):467-75.*
13. *Donadio G, Mensitieri F, Santoro V, Parisi V, Bellone ML, De Tommasi N, Izzo V, Dal Piaz F. Interactions with microbial proteins driving the antibacterial activity of flavonoids. Pharmaceutics. 2021 May 5;13(5):660.*
14. *Hua KF, Hsu HY, Su YC, Lin IF, Yang SS, Chen YM. Study on the anti inflammatory activity of methanol extract from seagrass Zostera japonica. Agric Food Chem. 2006,54:306-11.*
15. *Gokce G, Haznedaroglu MZ. Evaluation of antidiabetic, antioxidant and vasoprotective effects of Posidonia oceanica extract. J Ethnopharmacol. 2008;115:122-30.*
16. *Kannan RRR, Arumugam R, Anantharaman P. Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. Food Chem. 2012;135:2470-3.*
17. *Kumar SC, Sarada DVL, Gideon TP, Rengasamy R. Antibacterial activity of three south Indian seagrasses, Cymodocea serrulata, Halophila ovalis and Zostera capensis. World J Microbiol Biotechnol. 2008;24(9):1989-92.*
18. *Umamaheshwari R, Thirumaran G, Anantharaman P. Potential antibacterial activities from vellar estuary; south east coast of India. Adv Biomed Res.2009;3:140-3.*
19. *Natrah FMI, Harah ZM, Japar Sidik NI, Syahidah A. Antibacterial activities of selected seaweed and seagrass from port Dickson coastal water against different aquaculture pathogens. Sains Malays. 2015;44(9):1269-73.*
20. *Choi HG, Lee JH, Park HH, Sayegh FAQ. Antioxidant and antimicrobial activity of Zostera marina L. extract. Algae. 2009;24(3):179-84.*
21. *Sneka S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from Capparis decidua against Escherichia coli and Lactobacillus Species. Research Journal of Pharmacy and Technology. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773*
22. *Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22.*
23. *Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.*
24. *Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.*