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Seaweed Metabolites for Targeting Pel Polysaccharide Biosynthesis in *Pseudomonas aeruginosa*– A Novel Strategy for Biofilm Control

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Abstract

According to the National Institute of Health, more than 80% of microbial infections are biofilm-associated, including chronic lung, wound and ear infections. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes chronic nosocomial infection in immune-compromised and cystic fibrosis patients worldwide and is a common cause of urinary tract and burn infections. Its ability to form biofilm makes them highly resistant to antibiotics and the host's immune defense system. Biofilm produced by *Pseudomonas aeruginosa* is made up of exopolysaccharides such as pel, alginate and psl. Different strategies are reported in the control of biofilm formation. The present work focuses on targeting pellicle (pel) polysaccharide biosynthesis in *Pseudomonas aeruginosa* using a computational approach. Pel polysaccharide biosynthesis involves seven proteins present in the cytoplasmic, inner, periplasmic and outer membranes of *Pseudomonas aeruginosa*. In the current study, Pel D was taken as the target for inhibition of pel polysaccharide production. The marine environment provides a novel array of metabolites with unique structural features and bioactivities. Recently, metabolites derived from seaweeds or marine macroalgae have attracted increasing consideration for the development of novel drugs. In this perspective, thirty-nine metabolites reported in brown seaweed *Sargassum* were chosen as the source for druggable molecules (ligands). The structures of these metabolites were retrieved from the Seaweed Metabolite Database. Molecular docking was performed using PyRx software. Four metabolites of *Sargassum* were found to exhibit high binding affinity with target protein when compared to ibuprofen, a non-steroidal anti-inflammatory candidate drug reported for inhibition of biofilm control in *Pseudomonas aeruginosa*. The top four metabolites with high binding affinity were assessed for physicochemical, pharmacokinetic and drug-likeness properties through in silico analysis. Hence this study will pave the direction for exploring the potential of less explored seaweed metabolites as therapeutic mediators for the control of *Pseudomonas* biofilm infection.

Keywords: Seaweeds, *Sargassum sp*., *Pseudomonas aeruginosa*, Biofilm, Inhibitor, Pel D

Introduction

Microbes exist in two stages: free-living and aggregated. Free-living microbes adhere to living and non-living components and aggregate to form biofilms (1). According to the National Institute of Health, 80% of human infectious diseases are biofilm-associated infections (1).

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P. aeruginosa is a Gram-negative bacterium (2) that gives rise to nosocomial infections in immune-compromised patients and primary causative pathogen (3) for biofilm-associated pulmonary infections (4). *P. aeruginosa* causes infections with the help of virulence factors such as pili, lectins, alginate, EPS, lipopolysaccharide, and secreted virulence factors, namely, siderophores, cytotoxin, proteases, hemolysins, exotoxin A, exoenzyme U, exoenzyme S, etc. respectively (4). *Pseudomonas aeruginosa* biofilms form a protective barrier against antibiotics and the body's immune system (5). Biofilm matrix contains aggregated microbes that are in a protective polymeric matrix called extracellular polymeric substance (1) namely eDNA (extracellular DNA), polysaccharides and proteins (6). The biofilm makes the treatment difficult. Several strategies are proposed for Pseudomonal biofilm control such as adhesion inhibitors, biofilm maturation (communication) inhibitors, and promoters of disruption (1). Exopolysaccharides such as pel, psl and alginate facilitate the attachment, formation and stability of biofilm. In the initial stage of infection, psl and pel protect the *Pseudomonas aeruginosa* from the immune defense mechanism of the host. The growth of the biofilm of *Pseudomonas aeruginosa* is a result of nutrient depletion and the outcome is advantageous to the pathogen as it helps them to lower the metabolic activity (5). In this perspective, inhibition of the exopolysaccharide production by *Pseudomonas aeruginosa* using natural inhibitors would be a viable option to control biofilm formation, since polysaccharide is the major component of biofilm. Since pel is involved in antibiotic resistance and for the structure of biofilm (6) in the present work, pel exopolysaccharide biosynthesis was chosen for targeting the biofilm formation. The polysaccharide molecule plays a vital part in cell adhesion (6) and cross-links extracellular DNA. Through the action of the carbohydrate esterase Pel A in the periplasm, partial de-N-acetylation of pel polysaccharide occurs (7). In the biosynthesis of pel, the pel operon of *P. aeruginosa* has genes that encode seven protein complexes -pel (A, B, C, D, E, F and G). The precursors for the biosynthesis of pel come from central carbon metabolism and the other carbohydrates synthesis pathway (8).

Fig. 1 depicts the biosynthesis of pel in *Pseudomonas aeruginosa*. Pel D, E, G, and F form the synthase complex in the inner membrane and are involved in the synthesis and transport of pel into the periplasmic space (9). The TPR domain of Pel B localizes Pel A into the periplasmic space and both together are involved in the partial deacetylation of pel (10). Followed by deacetylation using Pel A, pel becomes positively charged. The polymer is attracted towards the electro-negatively charged concave surface of Pel C and directs the pel towards the exit channel formed by the beta-barrel domain of Pel B (11). Pel C customs a dodecameric funnel that mediates pel towards the Pel B pore (11). The mechanism of biofilm formation starts with the binding of c-di-GMP to the I-site of Pel D bringing in the structural conformational change (12). Then the three proteins of the inner membrane (Pel D, E, and G) are involved in the recruitment of the Pel F protein which localizes from the cytosolic to the inner membrane region (9). Pel F is a glycosyltransferase that polymerizes UDP-Gal NAc and UDP-GlcNAc to generate the pel polysaccharide. After which Pel A interacts with Pel B in the periplasm to partially deacetylate the polymer and this happens to produce a mature pel polysaccharide (13) that is eventually exported across the outer membrane along the β-barrel porin domain of Pel B (11). Biofilm formation is primarily controlled by cyclic- di-GMP. Biofilm formation and the adaptation of planktonic phenotypes are regulated by the higher and lower levels of cyclic- di-GMP respectively (14). The pel operon is activated only upon binding of c-di-GMP to the I-site of the degenerate GGDEF domain of the inner membrane Pel D, which is in the cytoplasmic part (15). Thus, through the inhibition of the c-di-GMP binding to the Pel D, the pel biosynthesis can be terminated thereby preventing the formation of biofilm. Fig. 2 represents the role

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of c-di-GMP in pel biosynthesis. Initial studies on the inhibition of the activity of Pel D can be executed using molecular docking analysis. Molecular docking studies are effective in the drug discovery process since it reduces the effort of scientists in wet lab studies. The ligands or candidate drug molecules with a high binding affinity towards the target protein can be chosen for further studies (16).

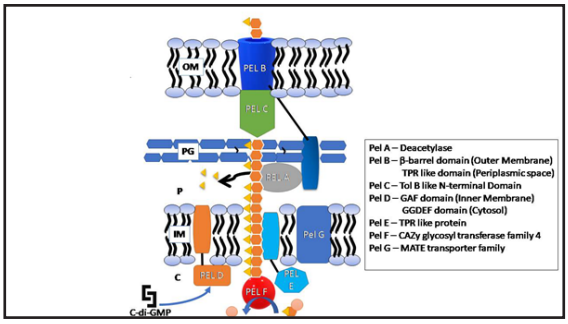

Fig. 1. Representation of Pel Biosynthesis in *Pseudomonas aeruginosa*.

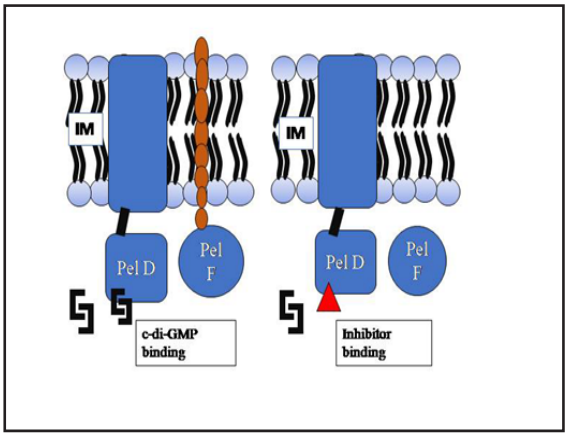

Fig.2. Pel Biosynthesis by c-di-GMP Binding and Inhibition accounted by the Binding of the Inhibitor to the Active Site.

Seaweeds are marine macroalgae that harbour a wholesome and abundant supply of bioactive substances, including vitamins, carotenoids, dietary fibers, proteins, and minerals [17]. Brown seaweed taken as a part of the diet was reported to lower the occurrence of cancer (17). Brown seaweed *Sargassum* was used to treat arteriosclerosis, skin diseases, liver organ swelling, scrofula neurosis, edema, angina pectoris, esophagitis, hypertension conditions, and chronic bronchitis (18). *Sargassum* is reported to possess biologically active metabolites that have immuno-modulatory, analgesic, anti-oxidant, neuroprotective, anti-microbial, fibrinolytic, anti-coagulant, anti-inflammatory, hepatoprotective, anti-tumor, and anti-viral activities (19). The inflammatory response was effectively suppressed by the aqueous extract of *Sargassum* (20). Therefore, the utilization of *Sargassum* species in the pharmaceutical and nutraceutical industries is highly promising. Thus, the metabolites from *Sargassum sp.* were used as a source of druggable molecules in the current study.

Materials and Methods

Retrieval of structures of target protein and ligands

Pel D was chosen as the target and the structure of Pel D (PDB id: 4dn0) was retrieved from RCSB-PDB. The energy of the protein structure was minimized for further studies (21). The structures of *Sargassum sp.* metabolites (to be used as a ligand) were retrieved from Seaweed Metabolite Database. The accession number referred to in the article corresponds to the accession number in Seaweed Metabolite Database.

Molecular docking of pel d with the metabolites of Sargassum

Molecular docking was performed using PyRx, an open-source tool for virtual screening of drugs and was used to study molecular interactions through a computational approach. To assess the binding affinity of the interacting ligands and the best ligand conformation for a particular target protein, molecular docking was performed. Binding scores are calculated in kcal/mol and the higher the negative value, the better binding of a ligand with the target protein, Pel D (22,23). Discovery studio was used to vi-

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sualize the interactions of the ligand with the target protein. Molecular docking was performed using PyRx software in Vina Wizard mode with ibuprofen as the standard drug with a search space of 14.932×25.12×20.88 Å, centered at (4.96, 3.11, -0.77). Ibuprofen was a non-steroidal anti-inflammatory candidate drug reported for inhibition of biofilm formation through anti-quorum sensing activity in *Pseudomonas aeruginosa* and was used as a standard drug (24).

Docking analysis and confirmation of interaction with active site of pel d

Analysis of docking was done from the data obtained from PyRx. The binding affinity of ligand conformation at RMSD 0 was chosen. Their interactions with the active site were confirmed by visualizing the ligand interaction with the target proteins obtained from molecular docking results in Discovery Studio (16).

Absorption, distribution, metabolism and excretion studies of ligands with top binding scores

Absorption, Distribution, Metabolism and Excretion (ADME) studies were performed using SwissADME, a web server (http://www.swissadme.ch/) and the physicochemical, drug-likeness, pharmacokinetics properties of the top binding seaweed metabolites were predicted (25). The physicochemical properties such as molecular formula, molecular weight, number of heavy atoms, number of H bond acceptor and donor, molar refractivity, Topological Polar Surface Area (TPSA), and solubility were computed. The pharmacokinetic properties like Gastrointestinal (GI) absorption, Blood Brain Barrier (BBB) permeability were assessed. The drug-likeness properties were evaluated by assessing the applicability of the Lipinski and Veber Rule. The ability of compounds to permeate through the Blood Brain Barrier and absorption of compounds through the human gastrointestinal tract was analyzed simultaneously using a BOILED-Egg plot (Brain Or IntestinaL Estimate D permeation). BOILED-Egg plot was obtained for top binding ligands using the SwissADME web server.

Results and Discussion

The results of molecular docking were depicted in Fig. 3 and Fig. 4 and the binding affinities of each metabolite with the target protein Pel D were tabulated in Table 1. Four metabolites of *Sargassum* were found to exhibit high binding affinity with target protein Pel D when compared to ibuprofen, a non-steroidal anti-inflammatory candidate drug reported for disruption of biofilm in *Pseudomonas aeruginosa.* Based on site-directed mutagenic studies, the active site residues of Pel D were reported to be ARG161, ARG367, ASP370, and ARG402 (12). The ligands (BS041, BS077, BS019 and BS061) with high binding affinity were found to interact with the active site residues of Pel D as visualized through the Discovery Studio (Table 2).

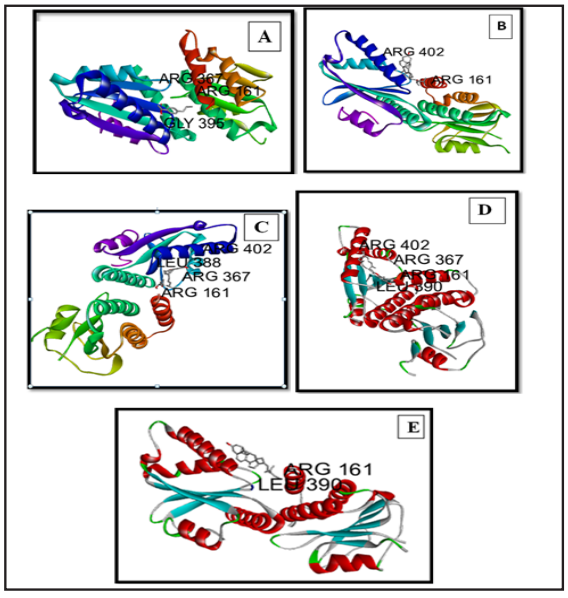

Fig. 3. 3D Visualization of Protein-Ligand Interaction using Discovery Studio; (A) Binding of Ibuprofen with Pel D (B) Binding of BS019 with Pel D (C) Binding of BS041 with Pel D (D) Binding of BS077 with Pel D (E) Binding of BS061 with Pel D.

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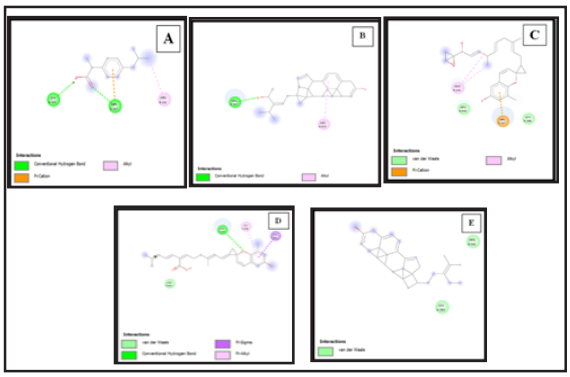

Fig 4: 2D Visualization of protein-ligand interaction through Discovery Studio (A) Ibuprofen interacting with amino acid residues of Pel D (B) BS019 interacting with amino acid residues of Pel D (C) BS041 interacting with amino acid residues of Pel D (D) BS077 interacting with amino acid residues of Pel D (E) BS061 interacting with amino acid residues of Pel D.

Physico-chemical, Pharmacokinetic and Drug likeness properties were evaluated (Table 3) and BS041 was found to possess better properties compared to others since it has a good number of hydrogen bond acceptors and donors. The following rules were proposed by Lipinski for determining the solubility and permeability of a compound to be tested as a candidate drug molecule: (1) the molecular mass should be less than 500 Dalton; (2) the hydrogen bond donor should be less than 5; (3) hydrogen bond acceptor should be less than 10 (4) Mlog P should be less than 4.15 (27). Out of the four top binding metabolites, BS041 satisfies all the rules whereas the rest of the 3 metabolites (BS077, BS019, BS061) have 1 violation of the Lipinski rule. The only violation is that it has an MLog P value greater than 4.15. However, 1 violation of the Lipinski rule is still acceptable (28) and thus all the compounds can be considered as druggable compounds. As per Veber's rule, the rotatable bonds should be less than 10 and the total polar surface area should be less than 140 sq. units (29, 30). All metabolites were found to possess one violation as per the Veber rule (Table 3).

Table 1 *Sargassum* Seaweed Metabolites retrieved from Seaweed Metabolite Database, their IUPAC name and Binding Affinity with Target Protein, Pel D

Ligands	IUPAC name	Binding Affinity (kcal/mol)
Ibuprofen	2-[4-(2-methylpropyl) phenyl] propanoic acid	-5.3
BS014	(6E,10E,14E)-2-hydroxy-2,6,10-trimethyl-14-[2-(5-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl) ethylidene]-3-oxopentadeca-6,10-dien-1-ylidyne	-5.8
BS015	(6E,10E,14E)-14-[2-(2,5-dihydroxy-3-methylphenyl) ethylidene]-2-hydroxy-2,6,10-trimethyl-3-oxopentadeca-6,10-dien-1-ylidyne	-6.3
BS016	18-{4-hydroxy-3-methyl-5-[(2E,6E,10Z)-11,14,15-trihydroxy-3,7,15-trimethylhexadeca-2,6,10-trien-1-yl]phenoxy}-18-oxooctadecylidyne	-5.6
BS017	(9Z)-18-{4-hydroxy-3-methyl-5-[(2E,6E,10Z)-11,14,15-trihydroxy-3,7,15-trimethylhexadeca-2,6,10-trien-1-yl] phenoxy}-18-oxooctadec-9-en-1-ylidyne	-5.2
BS018	(6E,10E,14E)-14-[2-(2,5-dihydroxy-3-methylphenyl) ethylidene]-2,3-dihydroxy-2,6,10-trimethylpentadeca-6,10-dien-1-ylidyne	-5.9
BS019	(2R,5S,14R,15R)-14-[(2R,4Z)-6-hydroxy-5-(propan-2- yl) hept-4-en-2-yl]-2,15-dimethyltetracyclo [8.7.0.0^ {2,7}.0^ {11,15}] heptadec-7- en-5-ol	-7.1
BS020	(2R,6R,14R)-6-hydroxy-2,14-dimethyl-13-[(5E)-5-(propan-2-yl) hept-5-en-2-yl]-5-oxatetracyclo [7.7.0.0^ {2,6}.0^ {10,14}] hexadecan-4- one	-6.3
BS049	2-methyl-6-[(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]cyclohexa-2,5-diene-1,4-dione	-5.5

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BS050	(2Z,6E,10E)-6,10-dimethyl-12-(5-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-2-(4-methylpent-3-en-1-yl) dodeca-2,6,10-trienoic acid	-5.7
BS046	(2Z,6E,10E)-12-(2,5-dihydroxy-3-methylphenyl)-6,10-dimethyl-2-(4-methylpent-3-en-1-yl) dodeca-2,6,10-trienoic acid	-5.8
BS047	(6E,10E,14E)-16-(2,5-dihydroxy-3-methylphenyl)-2-hydroxy-2,6,10,14-tetramethylhexadeca-6,10,14-trien-3-one	-6.2
BS048	2-[(2E,6E,10E,14R)-14,15-dihydroxy-3,7,11,15-tetramethylhexadeca-2,6,10-trien-1-yl]-6-methylbenzene-1,4-diol	-6
BS040	2-[(2E,6E,10E,14R)-14,15-dihydroxy-3,7,11,15-tetramethylhexadeca-2,6,10-trien-1-yl]-6-methylcyclohexa-2,5-diene-1,4-dione	-6.2
BS041	(3R,6E,10E)-13-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-2,6,10-trimethyltrideca-6,10-diene-2,3-diol	-7.1
BS042	2-[(2E,6E,10E)-12-hydroxy-11-(hydroxymethyl)-3,7,15-trimethylhexadeca-2,6,10,14-tetraen-1-yl]-6-methylbenzene-1,4-diol	-5.3
BS043	2-[(2E,6E,10E)-12-hydroxy-11-(hydroxymethyl)-3,7,15-trimethylhexadeca-2,6,10,14-tetraen-1-yl]-6-methylcyclohexa-2,5-diene-1,4-dione	-5.6
BS044	(2Z,6E)-2-(3-chloro-4-methylpent-4-en-1-yl)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methylnona-2,6-dienoic acid	-6.6
BS045	(2Z,6E)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methyl-2-(4-methylpent-3-en-1-yl) nona-2,6-dienoic acid	-6
BS051	(3E,5R)-1-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)- 4,8,12-trimethyltrideca-3,7,11-triene-5,6-diol	-6
BS052	2-[(2E,6E,10E)-8,9-Dihydroxy-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-yl]-6-methyl-1,4-benzoquinone	-6.2
BS053	(3E,5R,6R)-1-(6-methoxy-2,8-dimethyl-2H-chromen-2-yl)-4,8,12-trimethyltrideca-3,7,11-triene-5,6-diol	-6.3
BS061	(2R,5S,15R)-2,15-dimethyl-14-[(5Z)-5-(propan-2- yl) hept-5-en-2-yl] tetracyclo [8.7.0.0^ {2,7}.0^ {11,15}] heptadec-7- en-5-ol	-7
BS062	24-vinyl cholest-4-ene-3-one	-6.7
BS063	(3β)-Stigmasta-5,28-diene-3,24-diol	-6.6
BS064	(3S,5R,6S,3'S,5'R,6'R)-3,5'-dihydroxy-8-oxo-6',7'-didehydro-5,6-epoxy-5,6,7,8,5',6'-hexahydro-β, β-caroten-3'-yl acetate	-5.2
BS075	(2Z,6E)-2-(3,4-dihydroxy-4-methylpentyl)-9-(6- hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methylnona- 2,6-dienoic acid	-5.7
BS076	(2E,6E,10E)-6,10-dimethyl-12-(5-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-2-(4-methylpent-3-enyl) dodeca-2,6,10-trienoic acid	-5.7
BS077	(2Z,6E)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6- methyl-2-(4-methylpent-3-en-1-yl) nona-2,6-dienoic acid	-7.1
BS078	(2Z,6E)-2-(3,4-dihydroxy-4-methylpentyl)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methylnona- 2,6-dienoic acid	-6.1
BS079	(2E,6E)-2-(3,4-dihydroxy-4-methylpentyl)-7- [2- (furan-3-yl) ethyl] octa-2,6-dienedioic acid	-5.7
BS084	methyl2-(5-hydroxy-2-{[(6E,12R)-12-hydroxy- 3,7,11,15-tetramethyl-13-oxohexadeca-1,6,14-trien-3-yl] oxy} phenyl) acetate	-5.5
BS085	methyl 2-(5-hydroxy-2-{[(6E)-12-hydroxy-3,7,11,15- tetramethyl-13-oxohexadeca-1,6,14-trien-3- yl] oxy} phenyl) acetate	-5.6

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BS086	methyl2-(5-hydroxy-2-{[(6E,10E,13S)-13-hydroxy- 3,7,11,15-tetrameth-yl-12-oxohexadeca-1,6,10,14- tetraen-3-yl] oxy} phenyl) acetate	-5.7
BS087	methyl2-(5-hydroxy-2-{[(6E,10E,12S)-12-hydroxy- 3,7,11,15-tetrameth-yl-13-oxohexadeca-1,6,10,14- tetraen-3-yl] oxy} phenyl) acetate	-5.9
BS088	methyl2-(5-hydroxy-2-{[(6E,13E)-12-hydroxy-3,7,11,15-te-tramethylhexadeca-1,6,13,15-tetraen-3- yl] oxy} phenyl) acetate	-5.3
BS090	methyl2-{3-[(2E,6E,10E,12R,13S)-12,13-dihydroxy-3,7,11,15-tetramethyl-hexadeca-2,6,10,14-tetraen-1-yl]-2,5-dihydroxyphenyl} acetate	-6
BS092	methyl2-{2,5-dihydroxy-3-[(2E,10E,13S)-13-hydroxy-3,7,11,15-tetramethyl-6-oxohexadeca-2,10,14-trien-1-yl] phenyl} acetate	-6.3
BS093	(2Z,6E,10E)-12-(2,5-dihydroxy-3-methylphenyl)-6,10- dimethyl-2-(4-methyl-pent-3-en-1-yl) dodeca-2,6,10- trienoic acid	-5
BS094	(6E,10E)-16-(2,5-Dihydroxy-3-methylphenyl)-4,14-dihydroxy-2,6,10,14-te-tramethyl-2,6,10-hexadecatrien-5-one	-6.1

Table 2**:** Amino Acid Interaction at the Active Site of Pel D

Search Space (Angstrom): X 14.932, Y: 25.12 Z: 20.88; 6Centre X: 4.96, Y: 3.11, Z: -0.77	
Drug name/ Accession Number of Seaweed Metabo-lites	Amino Acid Interaction at the Active Site
Ibuprofen	ARG161, ARG367
BS019	ARG367, ARG402
BS041	ARG402
BS077	ARG161, ARG367, ARG402
BS061	ARG161

Table 3: Physico Chemical, Pharmacokinetic and Drug Likeness Properties of the Ligands with Top Binding Score

Properties	BS041	BS077	BS019	BS061
Molecular formula	$C_{27}H_{40}O_4$	$C_{27}H_{36}O_4$	$C_{29}H_{48}O_2$	$C_{29}H_{48}O$
Molecular weight (g/mol)	428.60	424.57	428.69	412.69
Number of heavy atoms	31	31	31	30
Number of H-bond acceptor	4	4	2	1
Number of H-bond donor	3	2	2	1
Molar refractivity	129.85	128.79	133.92	132.75
TPSA ($Å^2$)	69.92	66.76	40.46	20.23
MLog P	4.01	4.73	5.95	6.88
Solubility	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble
GI absorption	Low	Low	Low	Low
BBB permeability	No	No	No	No

Synthetic accessibility	6.31	6.15	6.31	6.15
Number of rotatable bonds	12	11	5	6
Violation of Lipinski rule	0	1	1	1
Violation of Veber rule	1	1	0	0

The BOILED egg plot analysis (Fig. 5) shows the solubility properties of the metabolites from *Sargassum sp.* The compounds that can permeate through the Blood Brain Barrier were represented in the yellow zone. The compounds that can be absorbed by the human gastrointestinal tract (HIA) were represented in the white zone. The compounds that can neither cross the BBB nor be absorbed by the HIA were represented in the grey zone. As evident from Fig. 5, compound BS041 was in the white zone and hence can be absorbed through the gastrointestinal tract. Metabolites BS077, BS019, and BS061 were found to be in the grey zone indicating that those molecules can neither cross the BBB nor be absorbed in the HIA. It will be difficult to synthesize these compounds chemically as the synthetic accessibility score was greater than 6 for all metabolites (BS041, BS077, BS019 and BS061).

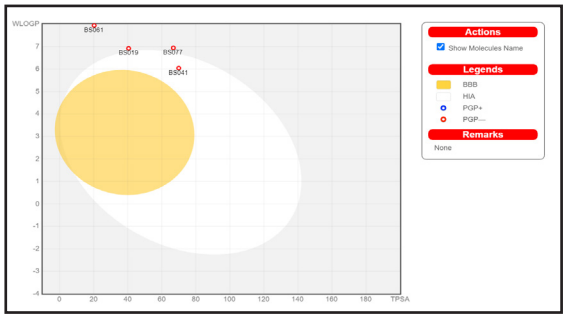

Fig 5: BOILED-Egg plot of BS019, BS041, BS061 and BS077 obtained from Swiss ADME.

Seaweed metabolites are unexplored molecules for therapeutic applications. In that context, using seaweed as a source of druggable molecules can open new possibilities for the treatment of biofilm-related infections. A combination of antibiotics has been attempted to disrupt biofilm formation, but it is not as effective in treating the infection due to the emergence of resistant strains. Until now, strategies were reported to prevent the formation of biofilm using various molecules targeting the proteins involved in quorum sensing [26]. But, inhibiting the production of pel polysaccharide is a novel approach to prevent the formation of biofilm. As the biofilm consists of 3 polysaccharides (pel, psl and alginate), pel is the one that plays a structural and protective role by providing resistance to aminoglycoside antibiotics in biofilm formation in *P. aeruginosa*. Further, it accounts for a cell-to-cell interaction. Hence manipulating the expression of pel was a suitable target for biofilm in *P. aeruginosa* (6). Inhibiting the formation of pel will destabilize the structure of biofilm, thus preventing the formation of biofilm. The pel biosynthesis pathway gets activated only upon the binding of c-di-GMP to the active site of Pel D, which undergoes a structural change and further activates other proteins (12). Thus, by the inhibition of this interaction, the production of pel can be stopped and thus averting biofilm formation. The ligands reported in Table 2 were found to have high binding affinity compared to others. By visualizing the interaction of these four ligand molecules, it is found that they bind to the active site of the protein. Therefore, these molecules can occupy the active site of Pel D, competitively preventing the binding of c-di-GMP. From the ADME studies, it was inferred that all molecules were not crossing the BBB barrier which is an attractive feature since the brain is not targeted. The rest of the properties are in the safe range. Also, the BOILED-Egg plot shows that BS041 has better human intestinal absorption compared to the rest. Hence the compound BS041, (3R, 6E, 10E)-13-(6-hydroxy-2, 8-dimethyl-2H-chromen-2-yl)-2, 6, 10-trimethyltrideca-6, 10-diene-2, 3-diol was found to be a better candidate due to its effective drug-likeness properties. The properties can further be improved through

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various approaches. Further in vitro and in vivo studies and validation experiments are necessary to confirm the anti-biofilm activity of the metabolites of *Sargassum sp.*

Conclusion

Seaweeds possess an immense number of bioactive molecules that are of therapeutic value. In this work, metabolites from brown seaweed *Sargassum* were used for the prevention of biofilm by *Pseudomonas aeruginosa* by in silico approach by targeting EPS production. Pel polysaccharide is one of the extra polymeric substances responsible for biofilm formation. Inhibition of pel production through the inhibition of the activator protein Pel D activity was attempted for the first time. From the present study, it can be concluded that compound BS041 from brown seaweed *Sargassum sp*. can serve as an effective drug molecule for inhibition of polysaccharide pel biosynthesis leading to suppression of biofilm formation by *P. aeruginosa*.

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