IBBJ

Winter 2018, Vol 4, No 1

Original Article

Phytochemical Characterization of Marine Macro Alga *Sargassum polycystem*, Molecular Docking, and *In Vitro* Anti-bacterial Activity against *Psuedomonas aeruginosa*

Gopalan Rajkumar[1], Periyakali Saravana Bhavan[1*], Muthu Suganya[1], Veeran Srinivasan[2], Madhayan Karthik[1], Rajendran Udayasuriyan[1]

1. Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India.

2. Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

Submitted 30 Sep 2017; Accepted 29 Oct 2017; Published 1 Dec 2017

Marine macro algae are useful to mankind as food, biofertilizer, and source of bioactive compounds such as agar-agar, alginates, and carrageenan. The aim of this study was to explore the phytochemicals, and the antibacterial activity of the marine alga *Sargassum polycystem*. The marine alga was subjected to ethanolic and methanolic extractions. The preliminary screening for phytochemicals showed the presence of tannins, polyphenols, saponins, cardiac glycosides, and quinones. The GC-MS analysis revealed the presence of totally 16 secondary metabolites: 8 different compounds for each solvent extraction. Among these bioactive compounds, 3 compounds (1, 2 benzenedicarboxylic-dibutylester and 13, docosenamide of ethanolic extract, and 3, 5-diaminodeoxymethoxy of methanolic extract, showed the binding affinity and ability to react with exotoxin-A of *Pseudomonas aeruginosa*, a common pathogenic bacterium of fishes and prawns. The *in vitro* antibacterial assays revealed that both ethanolic and methanolic extracts of *S. polycystem* possessed the ability to inhibit the growth of *P. aeruginosa*. Therefore, aquaculture medicine could be prepared with *S. polycystem*.

Keywords: Brown alga, ligands, exotoxin-A, *Sargassum polycystem*

Marine macro algae constitute a diverse group of organisms living for the benefit of mankind, are considered as ecologically and biologically important, contribute substantially to marine primary production, and provide a habitat for near shore benthic communities (1-2). They are classified as red (rhodophyta), brown (phaeophyta) or green (chlorophyta) algae depending on their nutrient, and chemical compounds. They contain many bioactive natural substances with medicinal properties such as antibiotics, antioxidant, anticoagulants, antiulcer etc., (3). Marine algae, apart from their valuable proteins carrying essential amino acids, dietary fiber, essential fatty acids, minerals and vitamins, are also a fine source of phenolic compounds (4, 5). *Sargassum* biomass is used as a potential renewable energy resource such as biogas (6-7). *Sargassum* has been used traditionally for treating scrofula, goiter, tumor, edema, testicular pain, and swelling (8). Algin, a carbohydrate present in *Sargassum* is used in textile, paper and pharmaceutical industries (9). The proximate composition of *Sargassum* species are varied from species to species. Based on their environments, they contain 12–16% proteins, and 1.5–2.0% lipids (10-12).

Herbal drugs may not only be useful as remedies, but also as growth stimulators, stress

*Correspondence: Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India. E-mail: bhavan@buc.edu.in

resistance boosters, and anti-infection agents (13-20). Hence, herbal drugs are achieving prosperity in disease management, because they are inexpensive, environmentally friendly, and have limited side effects. Several hundred plant varieties are used in medicine, and represent vital sources for effective drugs (21). People in developing countries rely on the traditional medicine for many diseases including cancer, congestive heart failure, hypertension, arthritis, asthma, and infections (22-25). Extracts of *S. polycystem* possess some medicinal properties, and have been tested against diabetes, acetaminophen-induced lipid peroxidation, hyperlipidemia, and stress in rats (26-28).

The antibiotics may reduce the larval growth and inhibit defense mechanisms of the host. Therefore, people are now turning towards natural products to avoid the adverse effects of antibiotics. Herbal extracts may control diseases due to their antioxidant and antimicrobial properties (29). Some natural plant products can exert medicinal activities like anti-depression, growth stimulation, appetite stimulation, immune stimulation, and antimicrobial properties in finned fishes and shrimps larvae (30).

In the present study, the brown alga, *Sargassum polycystem* was subjected to ethanolic and methanolic extractions in order to determine their phytochemicals. Further, the active principle compounds were subjected to molecular docking against the exotoxin-A of *Psuedomonas aeruginosa*, a common pathogenic bacterium of fishes and prawns in order to explore the binding, and interaction ability of ligands. Furthermore, the *in vitro* antibacterial activity of these extracts of *S. polycystem* was investigated against *P. aeruginosa*.

Materials and methods

Collection and identification of seaweed

The brown seaweed was collected from the intertidal region of Mandapam coast (Lat. 9° 17'N; Lon. 79° 19'E) of Gulf of Mannar, south-east coast of Tamil Nadu, India during September 2015. The macro alga species was identified as *S. polycystem* based on its morphology by using identification manual of "Economically Important Seaweeds" (31). Finally, the species, *S. polycystem* was authenticated by Dr. M. Palanisamy, Scientist 'C', Southern Regional Centre, Botanical Survey of India (BSI), Coimbatore, India.

Preparation of *S. polycystem* extracts

The collected *S. polycystem* was cleaned properly with seawater to remove all the foreign materials such as epiphytes, sand particles and necrotic parts, and brought to the laboratory in plastic bags. The sample was then rigorously washed with freshwater, sponged up, and spread to dry at room temperature for 2 weeks. Shade dried *S. polycystem* was ground to fine powder, and stored in sterilized containers for further usage.

The powdered sample of *S. polycystem* (75 g) was packed in a pouch made up of No. 1 Whatman filter paper, and ethanolic as well as methanolic extractions were performed (450 ml, 1:6 w/v) individually/ freshly for 6-9 h each (30 to 36 cycles) until a clear colorless solution was obtained by using Soxhlet apparatus. The extract was filtered by using double layer muslin cloth, concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP) connected with a vacuum pump and cryostat, and dried at 40 °C under hot air oven. The dark, gummy solid obtained was used for further investigations.

Qualitative analysis of *S. polycystem*

The ethanolic and methanolic extracts of *S. polycystem* were subjected to phytochemical analysis by adopting the standard qualitative procedures (32). The presence of different primary phytochemical compounds such as alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides, and quinones was detected.

Gas chromatography-mass spectrum (GC-MS) analysis

The extracts of *S. polycystem* were subjected to GC-MS (The Trace GC Ultra and DSQII model MS with inbuilt pre-filter to reduce the neutral particles, Thermo Fisher Scientific Company Pvt. Ltd.)

analysis for identification of different secondary phytochemical components with following working conditions: the injector port temperature was 250 °C; the interface temperature was 250 °C with source maintained at 200 °C; the oven temperature was programmed as variable, 70 °C for 2 min, 150 °C @ 8 °C/min, up to 260 °C @ 10 °C/min; the injector was operating in splitless mode; the DB-35ms nonpolar column (Agilent Co., USA) was 0.25 mm OD x 0.25 µm ID x 30 m length; helium was used as carrier gas at 1 ml/min; mass range scan: 50-650 Da; motor vacuum pressure was <40 bar; ionization energy was -70 eV.

The secondary phytochemical components present in each extract were identified by comparison of retention indices and mass spectra fragmentation patterns with those stored on the computer library, and also with published literature. National Institute of Standard and Technology (NIST4), and WILEY9 (33) on-line library source were also used for matching the identified components.

Isolation and characterization of *Pseudomonas aeruginosa* from infected freshwater *Macrobrachium rosenbergii* prawn, collected from a culture pond

Pseudomonas aeruginosa is a common pathogenic bacterium in aquaculture, and this disease causing agent led to black spot, brown spot, shell disease (melanized lesions, and bacterial necrosis) in all life stages, more commonly the juvenile and adult stages, of the freshwater prawn *Macrobrachium rosenbergii* (34).

The gram negative bacterium, *P.aeruginosa* was isolated from infected *M. rosenbergii* taken from a culture pond at Singanallur (Lat.10.99°N; Lon. 77.02°E), Coimbatore, Tamil Nadu, India, and brought to the laboratory in insulated box with ice to maintain a temperature around 4–6 °C. Affected muscle sample was cut, and homogenized with phosphate buffer saline (PBS), spread out into nutrient agar medium (Hi-Media), incubated at 37 °C for 24 h. Then bacterial colonies were observed, and isolated. The isolated colony was pure cultured into *Pseudomonas* isolation agar (Hi-Media), a specific medium for selective isolation of *Pseudomonas sp.*, incubated at 37 °C for 24 h. The isolated bacterial species was identified by morphological and biochemical characteristics. Molecular identification were previously reported and their GenBank accession number (KX398049) from our laboratory (35).

Molecular docking

Intermediary steps, such as PDBQT files for protein (bacterial toxin) and ligands (bioactive compounds identified) preparation and grid box creation were done by using graphical user interface program AutoDock Tools (ADT). ADT assigned polar hydrogens, united atom Kollman charges, solvation parameters, and fragmental volumes to the protein. AutoDock saved the prepared file in PDBQT format. Auto Grid was used for the preparation of the grid map using a grid box. The grid size was set to 60 × 60 × 60 xyz points with grid spacing of 0.375 Å and grid center was designated at dimensions (x, -1.095; y, -1.554 and z, 3.894). A scoring grid was calculated from the ligand structure to minimize the computation time.

AutoDock was employed for docking using protein and ligand information along with grid box properties in the configuration file. AutoDock uses the iterated local search global optimizer (36-37). Protein and ligands are asteemed as rigid. The results less than 1.0 Å in positional root-mean square deviation (RMSD) were clustered together, and represented by the most favorable free energy of binding. The pose with the lowest binding energy or binding affinity was extracted, and aligned with receptor structure for further analysis.

Determination of *in vitro* antibacterial activity

The antibacterial activity was studied by agar well diffusion method (38). To activate the bacterium before inoculation, it was cultured on nutrient broth (Hi-Media), and incubated at 37 °C for 24 h. For inoculum preparation, and antibacterial activity assay, Mueller-Hinton agar (Hi-Media,

India) was used. Approximately, 20 ml of the autoclaved medium was dispensed into sterile plates, and allowed to solidify under aseptic conditions. Then, the bacteria were inoculated, and spread with a sterile swab on the surface of agar plates. Briefly, three wells of 5 mm diameter were aseptically made on the assay plates seeded with target microorganism, using the wide end of sterile 200 µl pipette tips. The crude extracts of *S. polycystem* were reconstituted in respective solvents at a concentration of 100 mg/ml. The wells were then filled with 50 µl of each solvent extract of *S. polycystem*. The individual solvents (ethanol and methanol), and amoxicillin (250 mg/ml) were used as negative and positive controls, respectively. The plates were incubated overnight at 37 ºC. The diameter of inhibition zones, including the diameter of the well (5 mm) was measured, and compared to that of positive and negative controls.

Results

Phytochemicals of *S. polycystem*

The preliminary phytochemicals of *S. polycystem* extracts are presented in table 1. The ethanolic extract of *S. polycystem* showed the presence of 5 primary compounds, such as tannins, polyphenols, saponins, cardiac glycosides, and quinones. Of which polyphenols, saponins, cardiac glycosides and quinones were luxuriantly present. Tannins were moderately present. Similarly, the methanolic extract of *S. polycystem* showed the presence of 5 primary compounds, such as tannins, polyphenols, saponins, cardiac glycosides and quinones. Of which polyphenols, cardiac glycosides and quinones were luxuriantly present. Saponins were moderate, and tannins were just present in methanolic extract of *S. polycystem* (Table 1).

Table 1. The primary phytochemicals present in ethanolic and methanolic extracts of *S. polycystem*

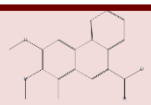
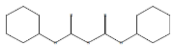
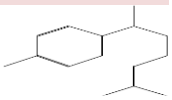
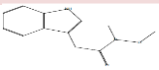
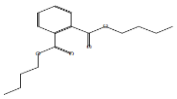
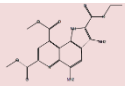
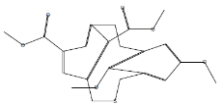
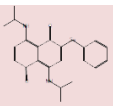
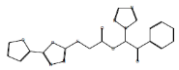
Phytochemicals	Different polar solvents	
	Ethanol	Methanol
Alkaloids	--	--
Terpenoids	--	--
Flavonoids	--	--
Tannins	++	+
Polyphenols	+++	+++
Saponins	+++	++
Cardiac gylcosides	+++	+++
Quinones	+++	+++

+: poor presence; ++: moderate presence; +++: luxuriant presence; --: absence.

GC-MS analyzes of the ethanolic extract of *S. polycystem* revealed the presence of 8 different secondary compounds {2, 3-dimethoxy-1-methyl-phenanthrene-9-carboxal-dehyde-formyl-d; tetradcane; hexadecane; octadecane; 1, 2-benzene dicarboxylic acid, dibutyl ester; 4-ethylene-3, 5-difiuro-2, 6-bis (trimethylsily)-4H-cyclopenta [2,1-b:3-4-b'] dithiophene; rac-4, 4' di-tert-butyl-6'hydroxymethyl-2,3,2',3'-tetraamethoxy-6-methyl-biphenol; 13 docosenamide, (Z)}, of which 4 compounds including tetradecane; hexadecane; 1, 2-benzenedicarboxylic acid, dibutyl ester; 13-docosenamide, (Z) showed bioactive properties (Tables 2 and 3; figure 1).

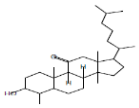
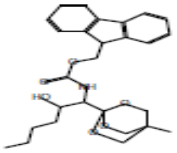
The methanolic extract of *S. polycystem* contained 8 different secondary compounds {13,18-î-epoxy-1-methyl-13, 17-seco-oestra-1, 3, 5(10)-trien-17-nitrile; thiodicarbonoate O, O-dicyclo-hexylester; benzene, 1-(1, 5-dimethyl-4-hexenyl)-4-methyl-; N-Methoxy-N-methyl-2-(indol3'yl) acetamide; 3, 5-diaminodeoxymethoxatin trimester; 6-Morpholino-2-(1-methylpyrazino) -17-hydroxy-androst-4-en-3-one 17-acetate; e-[5-(2-furyl)-1, 3, 4-oxadiazol-2-thiolacetoxy]e (1H1,2,4triazol1yl) acetophenone; 1-[N-(9-fluorenylmethyloxy-carbonyl)-(1S,2R)-1-amino-2-hydroxyhexyl]-4-methyl-2, 6, 7-trioxabicyclo [2.2.2] octane}, of which 4 compounds including 13,18-i-epoxy-1-methyl-13,17-seco-oestra-1,3,5(10)-trien-17-nitrile; N-methoxy-N-methyl-2-(indol3'yl) acetamide; 3,5-diaminodeoxymethoxy; 6-morpholino-2-(1-methyl-pyrazino) -17-hydroxyandrost-4-en-3-one 17-acetate contained bioactive principles (Tables 2 and 4;

Table 2. Secondary phytochemical compounds identified through GC-MS in ethanolic and methanolic extracts of *S. polycystem*, and their chemical structures and molecular formulae

Peak RT	Solvents			
	Ethanol		Methanol	
	Name of the compounds identified	Chemical structure and molecular formula	Name of the compounds identified	Chemical structure and molecular formula
4.73	2,3-Dimethoxy-1-methylphenanthrene-9-carboxaldehyde-formyl-d	 $C_{18}H_{15}DO_3$	--	--
7.77	Tetradecane	 $C_{14}H_{30}$	--	--
8.75	--	--	13,18-1-Epoxy-1-methyl-13,17-seco-oestra-1,3,5(10)-trien-17-nitrile	 $C_{19}H_{23}NO$
10.40	--	--	Thiodicarbonoate O,O-dicyclohexyl ester	 $C_{14}H_{22}O_2S_3$
10.74	Tetradecane	$C_{14}H_{30}$	--	--
12.41	--	--	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	 $C_{15}H_{22}$
14.84	Hexadecane	$C_{16}H_{34}$	--	--
17.51	--	--	N-Methoxy-N-methyl-2-(indol-3'yl) acetamide	 $C_{12}H_{14}N_2O_2$
19.10	Octadecane	$C_{22}H_{43}NO$	--	--
22.21	1,2-Benzenedicarboxylic acid, dibutyl ester	 $C_{16}H_{22}O_4$	--	--
23.17	--	--	3,5-diaminodeoxymethoxatin trimester	 $C_{18}H_{18}N_4O_6$
28.47	4-Ethylenedioxy3, 5difluoro2,6bis (trimethylsilyl)-4H-cyclopenta[2,1-b:3,4-b']dithiophene	 $C_{17}H_{22}F_2O_2S_2Si_2$	--	--
29.02	-- --		6-Morpholino-2-(1-methylpyrazino)-17-hydroxy and rost-4-en-3-one 17-acetate	 $C_{30}H_{47}N_3O_4$
31.81	-- --		e[5-(2-Furyl)-1,3,4-oxadiazol-2-thiolacetoxy]-e-(1H-1,2,4-triazol-1-yl)acetophenone	 $C_{18}H_{13}N_5O_5S$

RT: retention time

Table 2 Cont. Secondary phytochemical compounds identified through GC-MS in ethanolic and methanolic extracts of *S. polycystem*, and their chemical structures and molecular formulae

Peak RT	Solvents			
	Ethanol		Methanol	
	Name of the compounds identified	Chemical structure and molecular formula	Name of the compounds identified	Chemical structure and molecular formula
32.12	rac-4,4'-Di-tert-butyl-6'-hydroxymethyl-2,3,2',3'-tetramethoxy-6-methylbiphenol	 $C_{26}H_{38}O_5$	--	--
35.31	13-Docosenamide, (Z)-	 $C_{22}H_{43}NO$	--	--
36.29	--	--	1-[N-(9-Fluorenylmethyloxycarbonyl)-(1S,2R)-1-amino-2-hydroxyhexyl]-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane	 $C_{27}H_{33}NO_6$

RT: retention time

figure 2). Therefore, *S. polycystem* contains 16 different secondary compounds, of which 8 have bioactive properties (Tables 3 and 4).

Molecular docking

The 8 bioactive compounds were subjected to molecular docking with the exotoxin-A of *P. aeruginosa*. The exotoxin-A was found to be able to bind with three compounds only (2 active compounds from ethanolic, and 1 compound from methanolic extract); they were 1, 2 benzenedicarboxylic-dibutylester; 1, 3 docosenamide and 3, 5 diaminodeoxymethoxy, respectively. The ligand, 1, 2 benzenedicarboxylic- dibutylester was found to be able to bind its amino acid S113 to T533 of exotoxin-A. The second compound 1, 3 docosenamide was able to bind T533 (amino acid of protein) to S484 (amino acid of ligand). The third compound 3, 5 diaminodeoxymethoxy was able to bind K71 (amino acid of protein) and K485 (amino acid of ligand). The gliding score, docking score, and length of the H bond were shown in tables 5 and 6, and figures 3-5. The ethanol extracted compound of *S. polysytem* 1, 2 benzenedicarboxylic- dibutylester showed better gliding score (-3.8), followed by other compounds viz, 1, 3 docosenamide (-2.4), and 3, 5 diaminodeoxymethoxy (-2.3). These results confirmed the antibacterial activity of the extracted compounds against *P. aeruginosa*.

In vitro* antibacterial activity of *S. polycystem* extracts against *P. aeruginosa

The active components present in the seaweed *S. polycystem*, and extracted by ethanol and methanol showed antibacterial activity against the bacterium *P. aeruginosa*. This was assessed by measuring the zone of inhibition, and compared with the standard antibiotic, amoxicillin. The maximum inhibition zone of 13 mm was observed in ethanolic extract of *S. polycystem*, while the methanolic extract produced only 12 mm zone of inhibition. The positive control (amoxicillin) was produced 15 mm zone of inhibition. In the cases negative controls (ethanol and methanol), there were no inhibition zones (figure 6).

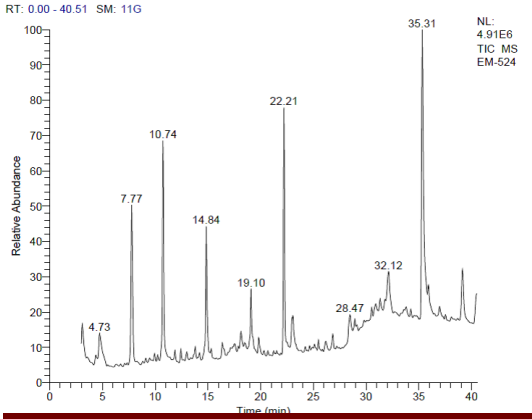


Figure 1. GC-MS chromatogram of the ethanolic extract of *S. polycystem*.

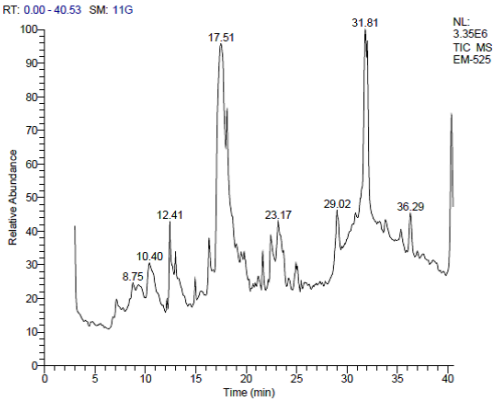


Figure 2. GC-MS chromatogram of the methanolic extract of *S. polycystem*.

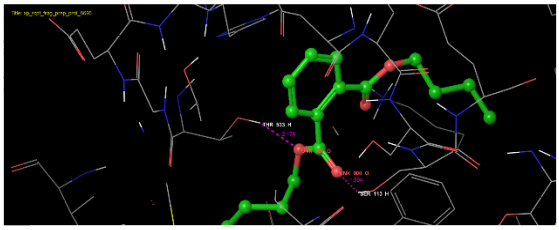


Figure 3. Complexity of protein–ligand interactions of exoto-xin-A with 1, 2 benzenedicarboxylic- dibutylester.

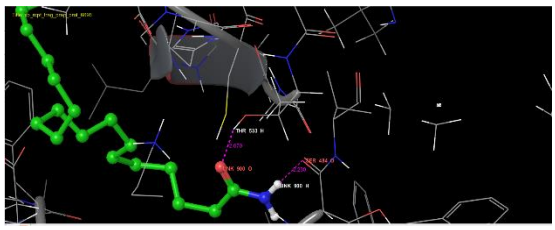


Figure 4. Complexity of protein–ligand interactions of exoto-xin-A with 1, 3 docosenamide.

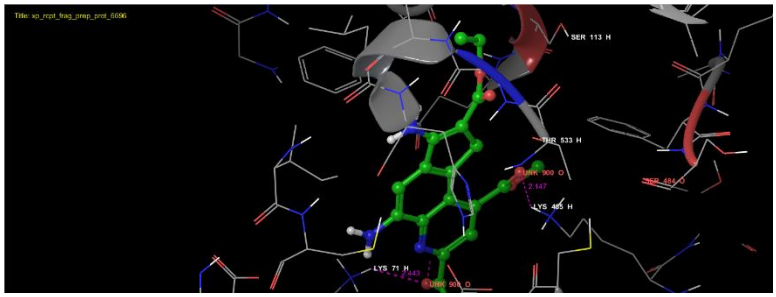


Figure 5. Complexity of protein–ligand interactions of exoto-xin-A with 3, 5-diaminodeoxymethoxy.

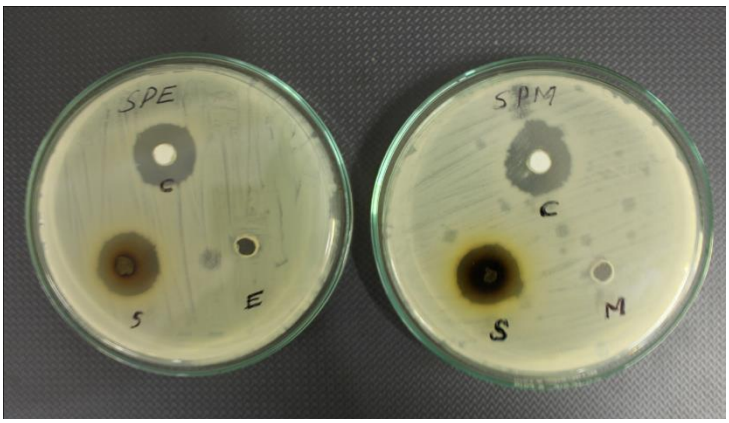


Figure 6. *In vitro* antibacterial activity of ethanolic and methanolic extracts of *S. polycystem* against *P. aeruginosa*. SPE: *Sargassum polycystem* ethanolic extract; SPM: *Sargassum polycystem* methanolic extract; C: positive control (amoxicillin); S: sample; E and M: negative control (ethanol and methanol).

Discussion

In the present study, the ethanolic extract of S. polycystem showed moderate presence of tannins, whereas methanol extract of S. polycystem showed just presence of tannins. The presence of tannins, polyphenols and cardiac glycosides was reported in methanolic extract of *S. polycystem* and *T. ornata* (39-40). Tannins are used in medicine as constrictive agents, healing factors in inflammation, leucorrhoea, gonorrhea, hurts, clumps, and as antitoxins. Tannins also showed inhibitory effects against viruses, bacteria, and parasites. Their antioxidant and anti-ulcer properties make them favorite candidatesfor possible therapeutic applications (41-42).

In the present study, ethanolic and methanolic extracts of S. polycystem showed luxuriant presence of polyphenols. The phenolic compounds, have biological and pharmacological properties especially antimicrobial, anti-viral, anti-inflammatory, cytotoxic, anti-mutagenic, and anti-carcinogenic activities (43-45). Seaweeds extracts are considered to be a rich source of phenolic compounds (46-47). Phenolic compounds are one of the most effective antioxidants in brown algae (48).

The ethanolic extract of S. polycystem showed luxuriant presence, whereas methanolic extract showed moderate presence of saponins. Saponins are involved in various biological effects including hemolytic and anti-bacterial activities (49).

In the present study, ethanolic and methanolic extract of *S. polycystem* showed luxuriant presence of cardiac glycosides. Cardiac glycosides are one of the most prevailing phytoconstituents, and are used as arrow poisons or cardiac drugs (50). The cardiac glycosides are steroid compounds which act specifically and mainly on the cardiac muscle upon injection into man or animals. They are used as agent of choice in the treatment of congestive cardiac failure (51). The ethanolic and methanolic extracts of *S. polycystem* showed also luxuriant presence of quinones. Quinones are often used in the treatment of malaria and tumors (52). They have anti-inflammatory, anti-bacterial, and immunomodulating potentials (53).

Table 3. GC-MS profiles of phytochemicals for ethanolic extracted *S. polycystem* components

RT	Name of the compounds	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
4.73	2,3-Dimethoxy-1-methylphenanthrene-9-carboxaldehyde-formyl-d	31.81	$C_{18}H_{15}DO_3$	280	3.32	472	663	---
7.77	Tetradecane	8.45	$C_{14}H_{30}$	198	10.59	754	843	Antimicrobial (54)
10.74	Tetradecane	46.32	$C_{14}H_{30}$	198	11.47	911	922	Antimicrobial (54)
14.84	Hexadecane	35.11	$C_{16}H_{34}$	226	8.40	894	905	Antibacterial, antioxidant (55-56)
19.10	Octadecane	30.72	$C_{18}H_{38}$	254	3.57	876	906	---
22.21	1,2-Benzenedicarboxylic acid, dibutyl ester	20.11	$C_{16}H_{22}O_4$	278	12.37	933	967	Antimicrobial, antifouling (57)
28.47	4-Ethylenedioxy-3,5-difluoro-2,6-bis(trimethylsilyl)- 4H –cyclopenta [2,1-b:3,4-b'] dithiophene	88.28	$C_{17}H_{22}F_2O_2S_2Si_2$	416	1.97	800	802	---
32.12	rac-4,4'-Di-tert-butyl-6'-hydroxymethyl-2,3,2',3'-tetr amethoxy-6-methylbiphenol	95.93	$C_{26}H_{38}O_5$	430	4.72	694	721	---
35.31	13-Docosenamide, (Z)-	50.87	$C_{22}H_{43}NO$	337	19.79	638	694	Antimicrobial (58)

RT: retention time; P: probability; MF: molecular formula; MW: molecular weight; SI: similar index; RSI: reverse similar index; --- - absent activity.

Table 4. GC-MS profiles of phytochemicals for methanolic extracted *S. polycystem* components

RT	Name of the compound	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
8.75	13,18-i-Epoxy-1-methyl-13,17-seco-oestra-1,3,5(10)-trien-17-nitrile	25.46	$C_{19}H_{23}NO$	281	0.83	446	623	Analgesic (59)
10.40	Thiodicarbonoate O,O-dicyclohexylester	64.90	$C_{14}H_{22}O_2S_3$	318	4.91	458	786	---
12.41	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	68.47	$C_{15}H_{22}$	202	4.44	859	965	---
17.51	N-Methoxy-N-methyl-2-(indol3'yl)acetamide	18.70	$C_{12}H_{14}N_2O_2$	218	28.34	428	937	Antioxidant, anti-inflammatory (60, 61)
23.17	3,5-diaminodeoxymethoxatin trimester	73.34	$C_{18}H_{18}N_4O_6$	386	7.08	701	866	Antibacterial (62)
29.02	6-Morpholino-2-(1-methylpyrazino)-17-hydroxyandrost-4-en-3-one 17-acetate	93.19	$C_{30}H_{47}N_3O_4$	513	4.28	795	923	Antibacterial (63-64)
31.81	e-[5-(2-Furyl)-1,3,4-oxadiazol-2-thiolacetoxy]e (1H1,2,4triazol1yl)acetophenone	9.67	$C_{18}H_{13}N_5O_5S$	411	18.23	328	467	---
34.34	1-[N-(9-Fluorenylmethyloxycarbonyl)-(1S,2R)-1-amino-2-hydroxyhexyl]-4-methyl-2,6,7-trioxabicyclo [2.2.2]octane	21.17	$C_{27}H_{33}NO_6$	467	2.97	365	700	---

RT: retention time; P: probability; MF: molecular formula; MW: molecular weight; SI: similar index; RSI: reverse similar index; --- - absent activity.

Table 5. Details of glide score, docking score, and hydrogen bond length for ligands and exotoxin-A protein

Name of the compound	Glide score	Docking score	H bond
1,2-Benzenedicarboxylic acid, dibutyl ester	-3.8	-3.8	-1.1
13-Docosenamide	-2.4	-2.4	-1.1
3, 5 Diaminodeoxymethoxy	-2.3	-2.3	-0.5

Table 6. Details of glide score, docking score, and hydrogen bond length for ligands and exotoxin-A protein

Name of the compounds	AA from exotoxin-A	AA from ligand	2ZIT atom	Bond length
1,2-Benzenedicarboxylic acid, dibutyl ester	Hydrogen	Oxygen	T533	2.176
	Oxygen	Hydrogen	S113	1.994
13-Docosenamide	Hydrogen	Oxygen	T533	2.070
	Oxygen	Hydrogen	S484	2.230
3, 5 Diaminodeoxymethoxy	Hydrogen	Oxygen	K71	2.443
	Hydrogen	Oxygen	K485	2.147

Regarding the antibacterial activity, the ethanolic extract of *S. polycystem* showed the strongest activity against *P. aerugionsa*. In *P. aeruginosa*, exotoxin-A exerts its cellular toxicity through ADP ribosylation of translation elongation factor 2, this finally results into enzyme cleavage activity and binding of cell surface receptor and this can cause toxicity in infected cell, hence the target is important (61). The compounds, 1, 2 benzenedi-carboxylic- dibutylester, 1, 3 docosenamide, and 3, 5 diaminodeoxymethoxy showed a great anti-bacterial activity against exotoxin-A.

The seaweeds are rich in secondary metabolites with great medicinal values, and have been largely used in the drug and pharmaceutical fabrication.

Many of the seaweeds possess bioactive components, which inhibit the growth of some gram-positive and gram-negative bacteria (65). In general, the marine algal extracts were used as remedies against helminths, bacteria, fungi, cough, hypertension, tumor, and diarrhea (65-67).

In conclusion, the GC-MS analysis revealed the presence of 16 phytochemical components. Of which 8 compounds were previously reported to have bio active properties. Among the 8 bioactive compounds, 3 showed affinity to interact with exotoxin-A of *P. aeruginosa*. These three active principle compounds need to be isolated, purified and characterized first and then a detailed study can be conducted on their pharmaceutical properties.

Acknowledgement

The authors gratefully acknowledge Dr. M. Palanisamy, Scientist 'C', Southern Regional Centre, Botanical Survey of India (BSI), Coimbatore, India, for authentication of the seaweed, *Sargassum polycystem*. The South India Textile Research Association (SITRA), Coimbatore, Tamil Nadu, India, is acknowledged for providing GC-MS outsourcing service.

Conflict of interest

The authors declare that they have no competing interest.

References

1. Mann K H. Seaweeds: Their Productivity and Strategy for Growth. Science. 1973;182:975-81.

2. Williams S L and Smith J E. A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annu Rev Ecol Evol Syst. 2007;38:327-59.

3. Lekameera R, Vijayabaskar P, Somasundaram S. Evaluating antioxidant property of brown alga Colpomenia sinuosa (Derb. Et sol). Afri J Food Sci. 2013;2:126-30.

4. Misurcova L. Chemical composition of seaweeds. In Handbook of Marine Macroalgae: Biotechnology and Applied Phycology; Kim, SK., Ed.; John Wiley & Sons: Chichester, UK, 2011; 173-192.

5. Ambrozova J V, Misurcova L, Vicha R, et al. Influence of extractive solvents on lipid and fatty acids content of edible freshwater algal and seaweed products, the green Microalga Chlorella kessleri and the Cyanobacterium Spirulina platensis. Molecules. 2014;19:2344-60.

6. Yokoyama S, Jonouchi K, Imou K. Energy production from marine biomass: fuel cell power generation driven by methane produced from seaweed. Proc World Acad Sci Eng Tech. 2007;22:320-3.

7. Bhaigyabati T, Usha K. Preliminary phytochemical screening and antioxidant activity of various extracts of Sargassum wightii greville. Int J Uni Pharm Biol Sci. 2007;2(6):60-69.

8. Kandale A, Meena A, Rao M, et al. Marine algae: an introduction, food value and medicinal uses. J Pharm Res. 2011;4:219-21.

9. Wang S, Jiang X, Han X, et al. Combustion characteristics of seaweed biomass. 1. Combustion characteristics of Enteromorpha clathrata and Sargassum natans. Energy & Fuels. 2009;23:5173-8.

10. Kaliaperumal N, Chennubhotla V, Najmuddin M, et al. Biochemical composition of some common seaweeds from Lakshadweep. J Mar Biol Ass India. 1994;36:316-9.

11. Murugaiyan K, Narasimman S, Anatharaman P. Proximate composition of marine macro algae from Seeniappa Dharka, Gulf of Mannar region, Tamil Nadu. Int J Res Mari Sci. 2012;1:1-3.

12. Hemavijayan PU, George G. Phytochemical estimation of selected brown seaweeds from Mulloor coast, Kerala. Ind J App Res. 2015;5;134- 136.

13. Bhavan P, Jeyanthi S, Rebecca A. Growth performance of the freshwater prawn Macrobrachium rosenbergii post larvae fed

with Ocimum sanctum (tulsi) and Withania somnifera (ashwagandha) incorporated feeds. Int J Biol Res Dev . 2011;1:34-53.

14. Bhavan S P, Manickam N, Radhakrishnan S. Influence of herbal greens, Murraya koenigii, Coriandrum sativum and Menthe arvensis on growth performance of the freshwater prawn Macrobrachium rosenbergii post larvae. Res J Biotechnol. 2012;7:149-57.

15. Poongodi R, Bhavan P S, Muralisankar T, et al. Growth promoting potential of garlic, ginger, turmeric and fenugreek on the freshwater prawn Macrobrachium rosenbergii. Int J Pharma Bio Sci. 2012;3:914-26.

16. Bhavan P S, Saranya C, Manickam N, et al. Effects of Piper longum, Piper nigram and Zingiber officinale on survival, growth, activities of digestive enzymes and contents of total protein, vitamins and minerals in the freshwater prawn Macrobrachium rosenbergii. Elixir Bio Tech. 2013;58:14824-8.

17. Aarumugam P, Bhavan P S, Muralisankar T, et al. Growth of macrobrachium rosenbergii fed with mango seed kernel, banana peel and papaya peel incorporated feeds. Int J Appl Bio Pharma Tech. 2013;4(2):12-25.

18. Bhavan P S, Anisha T, Srinivasan V, et al. Effects of spices, Papaver somniferum, Elettaria cardamomum, Foeniculum vulgare and Syzygium aromaticum on growth promotion in Macrobrachium malcolmsonii early juveniles. Int J Pure App Biosci. 2014;2:120-31.

19. Bhavan P S, Mohammedsiddiq S, Srinivasan V, et al. Effects of seeds of medicinal plants, Syzygium cumini, Phylanthus emblica, Azadirachta indica and Ricinus communis on growth promotion in Macrobrachium malcolmsonii early juveniles. Int J Res Stud Biosci. 2014;2:95-106.

20. Dhanalakshmi K, Bhavan PS, Rajkumar G. et al. Phytochemical characterization of couch grass (Cynodon dactylon) and its growth promoting potential on the freshwater prawn Macrobrachium rosenbergii post-larvae. Brit Biotech J. 2016;14:1-24.

21. Pandey G, Madhuri S, Mandloi A. Medicinal plants useful in fish diseases. Plant Arch. 2012;12:1-4.

22. Mashour N H, Lin G I, Frishman W H. Herbal medicine for the treatment of cardiovascular disease: clinical considerations. Arch Intern Med. 1998;158:2225-34.

23. Lee K. Recent new drugs discovered and developed from Chinese medicine. Biopharma quarterly. 1998;4:12-5.

24. Lee K-H. Anticancer Drug Design Based on Plant-Derived Natural Products1. J Biomed Sci. 1999;6:236-50.

25. Lee K-H and Morris-Natschke S L. Recent advances in the discovery and development of plant-derived natural products and their analogs as anti-HIV agents. Pure Appl Chem. 1999;71:1045-51.

26. Motshakeri M, Ebrahimi M, Goh Y M, et al. Effects of brown seaweed (Sargassum polycystum) extracts on kidney, liver, and pancreas of type 2 diabetic rat model. J Evid Based Complementary Altern Med. 2014;2014:1-11.

27. Raghavendran H R, Sathivel A, Devaki T. Protective effect of Sargassum polycystum (brown alga) against acetaminophen-induced lipid peroxidation in rats. Phytother Res. 2005;19:113-5.

28. Raghavendran H R, Sathivel A, Devaki T. Effect of Sargassum polycystum (Phaeophyceae)-sulphated polysaccharide extract against acetaminophen-induced hyperlipidemia during toxic hepatitis in experimental rats. Mol Cell Biochem. 2005;276:89-96.

29. Ramudu K R and Dash G. A review on herbal drugs against harmful pathogens in aquaculture. Am J Drug Discov Dev. 2013;3:209-19.

30. Citarasu T. Herbal biomedicine: A new opportunity to aquaculture industry. Aquacult Int. 2010;18; 403-414.

31. Kaliaperumal N, Kalimuthu S, Ramalingam J R. Economically Important Seaweeds, special publication number 62, central marine fisheries research institute, Indian Council of Agricultural Research. 1995.

32. Trease G E, Evans W C. Pharmacology 11th Edn., Bailliere Tindall Ltd., London, 1989;60-75.

33. Van Den Dool H and Dec. Kratz P. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J Chromatogr A. 1963;11:463-71.

34. FAO. Cultured Aquatic Species Information Programme Macrobrachium rosenbergii (De Man, 1879). FAO Fisheries and Aquaculture Department. Aquatic Animal Pathogen and Quarantine Information System AAPQIS. 2014.

35. Srinivasan V, Bhavan P S, Rajkumar G, et al. Dietary Supplementation of Magnesium Oxide (MgO) Nanoparticles for Better Survival and Growth of the Freshwater Prawn Macrobrachium rosenbergii Post-larvae. Biol Trace Elem Res. 2017;177:196-208.

36. Baxter J. Local optima avoidance in depot location. J Oper

Res Soc. 1981;815-9.

37. Blum C, Roli A, Sampels M, Hybrid metaheuristics: an emerging approach to optimization. Studies in Computational Intelligence. Berlin Heidelberg: Springer-Verlag; 2008;114.

38. Patra J, Patra A, Mahapatra N, et al. Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. Mala J Micro. 2009;5:128-31.

39. Asha Kanimozhi S, Johnson M, Renisheya Joy Jeba Malar T. Phytochemical composition of Sargassum polycystum C. agardh and Sargassum duplicatum J. agardh. Int J Pharm Pharm Sci. 2012;7:393-7.

40. Rajkumar G and Bhavan P S. Phytochemical characterization of the marine brown alga Turbinaria ornata. Res J Chem Environ. 2017;21:54-63.

41. Wallace G and Fry S C. Phenolic components of the plant cell wall. Int Rev Cytol. 1994;151:229-67.

42. Ndhlala A, Kasiyamhuru A, Mupure C, et al. Phenolic composition of Flacourtia indica, Opuntia megacantha and Sclerocarya birrea. Food Chem. 2007;103:82-7.

43. Prior R L, Wu X, Gu L. Flavonoid metabolism and challenges to understanding mechanisms of health effects. J Sci Food Agri. 2006;86:2487-91.

44. Mungole a J, Awati R, Chaturvedi A, et al. Preliminary Phytochemical screening of Ipomoea obscura (L)-A hepatoprotective medicinal plant. Int J Pharm Res. 2010;2:2307-12.

45. Raja R D, Jeeva S, Prakash J W, et al. Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac J Trop Med. 2011;4:375-8.

46. Heo S J, Park E J, Lee K W, et al. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol. 2005;96:1613-23.

47. Duan X-J, Zhang W-W, Li X-M, et al. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. Food Chem. 2006;95:37-43.

48. Huang J, Liu Y, Wang X. Selective adsorption of tannin from flavonoids by organically modified attapulgite clay. J Haz Mat. 2008;160:382-7.

49. Sparg S G, Light M E, Van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol. 2004;94:219-43.

50. Firn R. Nature's Chemicals. Oxford University press, Oxford. 2010;74-75.

51. Tripathi K D. Cardiac glycosides and drug for chief essential of medicinal pharmacology, 4th Edition (Jay Pee Brothers medical publishers Pvt. Ltd. New Delhi) 2002;497.

52. Al-Khalil S, Alkofahi A, El-Eisawi D, et al. Transtorine, a new quinoline alkaloid from Ephedra transitoria. J Nat Prod. 1998;61:262-3.

53. Bergh J C S, Tötterman T H, Termander B C, et al. The First Clinical Pilot Study of Roquinimex (Linomide®) in Cancer Patients with Special Focus on Immunological Effects. Cancer Invest. 1997;15:204-11.

54. Gopinath S, Sakthidevi G, Muthukumaraswamy S, et al. GC-MS analysis of bioactive constituents of Hypericum mysorense (Hypericaceae). J Curr Chem Pharm Sci. 2013;3:6-15.

55. Kumar V, Bhatnagar A, Srivastava J. Antibacterial activity of crude extracts of Spirulina platensis and its structural elucidation of bioactive compound. . J Med Plants Res. 2011;5:7043-8.

56. Yogeswari S, Ramalakshmi S, Neelavathy R, et al. Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. Glob J Pharm. 2012;6:65-71.

57. Maruthupandian A and Mohan V. GC-MS analysis of some bioactive constituents of Pterocarpus marsupium Roxb. Int J Chem Tech Res. 2011;3:1652-7.

58. Ghazali N, Abdullah N A, Bakar A, et al. GC-MS analysis of some bioactive components in the root extract of Ixora coccinea linn. Int J Pharm Bio Sci. 2014;5:197-203.

59. Anonymous. National Health & Medical Research Council of Australia: Acute pain management Scientific evidence. Ausinfo, Canberra. 1990.

60. Oxenkrug G. Antioxidant Effects of N-Acetylserotonin. Ann N Y Acad Sci. 2005;1053:334-47.

61. Perianayagam M C, Oxenkrug G F, Jaber B L. Immune-modulating effects of melatonin, N-acetylserotonin, and N-acetyldopamine. Ann N Y Acad Sci. 2005;1053:386-93.

62. Geller B L. Antibacterial antisense. Curr Opin Mol Ther. 2005;7:109-13.

63. Deas T S, Bennett C J, Jones S A, et al. In vitro resistance selection and in vivo efficacy of morpholino oligomers against West Nile virus. Antimicrob Agents Chemother. 2007;51:2470-82.

64. Solis F J and Wets R J-B. Minimization by random search techniques. Mathematics of operations research. 1981;6:19-30.

65. Kolanjinathan K, Ganesh P, Govindarajan M. Antibacterial

activity of ethanol extracts of seaweeds against fish bacterial pathogens. Eur Rev Med Pharmacol Sci. 2009;13:173-7.

66. Pesando D and Caram B. Screening of marine algae from the French Mediterranean coast for antibacterial and antifungal activity. Botanica marina. 1984;27:381-6.

67. Yi Z, Yin-Shan C, Hai-Sheng L. Screening for antibacterial and antifungal activities in some marine algae from the Fujian coast of China with three different solvents. Chin J Ocean Lim. 2001;19:327-31.