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Research Article

STUDY ON PHYTO-CHEMICAL AND ANTIMICROBIAL ACTIVITY OF SOME SELECTED MARINE SEAWEEDS AGAINST HUMAN AND FISH PATHOGENS

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ABSTRACT

Seaweeds are used in pharmaceutical and biochemical applications as they possess interesting biological activities that contribute to the discovery of natural therapeutic agents. In the present work, we used four seaweeds (*Sargassum wightii, Ulva fasciata, cauterpa racemosa* and *Padina gymnospora*), extracted in five solvents (chloroform, methanol, petroleum ether, acetone and butanol) and tested for their antimicrobialactivty against 7 human bacterial pathogens (*Staphylococcus aureus, Streptococcus mutants, Bacillus subtilus, Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli* and *Salmonella typhimurium* and 6 fish bacterial pathogens (*Aeromonas sp , Pseudomonas sp, Flavobacterium sp, Yersinia sp,* and *Vibrio cholera*). All the extracts in all the seaweeds were highly effective against bacteria. All the extracted seaweeds contain tannin, phenol, saponins, alkaloids and flavanoids phytochemical compounds. In future, the research may help to preparation of bioactive nanopartical compounds.

Keywords; Marine algae, Human and fish bacterial pathogens, solvents, antibacterial activity

INTRODUCTION

Seaweeds are primitive on flowering plants without true root stem and leaves. They include one of the commercially important marine renewable living resources which are also used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals and dietary fibers[1]. Biostimulant properties of seaweeds are explored for use in the development of novel antibiotics. Seaweeds have some valuable medicinal compounds such as antibiotics, laxative, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Many metabolites isolated from marine algae have bioactive efforts ([2,3,4].

Among different compounds with functional properties, antioxidants are the most widely studied. Oxidative stress is an important factor in the pathological genesis, from cancer to cardiovascular and degenerative disease. several compounds from the algae show pharmacological activities and bioactive compounds, primarily for treating deadly diseases like cancer, acquired immuno deficiency syndrome, arthritis etc., while some compound have been used to treat inflammation [5]. Marine algae are continuously exposed too much biotic and abiotic pressure which influences the organism's physiology and in turn leads to the production of multifunctional natural secondary metabolites (SM). So far more than 2400 SM described and many of the SM is natural blueprints for the development of new drugs [6,7] .

Bacterial infection causes high rate of mortality in human population and aquaculture organisms [8]. Bacillus cereus is responsible for causing food born diseases. *Enterococcus faecalis* is the causative agent of inflammatory bowel disease. *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complication. The major secondary metabolites produced by seaweeds are halogenated compounds [9] displaying antibacterial, antifungal, antiviral, antifouling, and anti-feedent properties. Although thousands of bioactive compounds have been discovered the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms.

The nutritional property of seaweeds from some regions of the world and Indian coast has been well documented [10, 11, 12] Climate and sea conditions may cause variations in nutrient composition of seaweeds [13, 14] . The aim of the present investigation was to study the phyto-chemical compounds and develop the standard method for extraction of four species of seaweeds. The extracted compounds were employed their potential was reported against human and fish bacterial pathogens.

Materials and Methods

Collection of sample

A total of four seaweeds were collected from Mandapam coastal line, Rameswaram in Tamilnadu, India during September 2012. Seaweeds such as *Sargassum wightii, Ulva fasciata , Caulerpa racemosa* and *Padina gymnospora were* collected by handpicking. The samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles and shells then brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washing seaweeds were blotted on the blotting paper and spread out room temperature in shade. Shade dried sample were grounded into fine powder. The powdered samples were then stored in refrigerator for further use.

Preparation of extracts

The powdered samples (10g) and packed in Soxhlet apparatus and extracted with chloroform, methanol, petroleum ether, acetone and butanol for 8h. The solvent was evaporated from crude extract by rotatory evaporator. The dried extracts were dissolved in 2ml of respective solvents or sterile water and stored at 4^{o}C until use.

Phyto-chemical study

The preliminary phyto-chemical compounds such as Carbohydrate, proteins, fats, alkaloids, steroids, tannin, phenols, saponins, gum and flavonoids were analyzed by Harborne methods [15] .

Antibacterial activity test

Test organisms:

Human pathogens

The bacterial human pathogen strains *Staphylococcus aureus, Escherichia coli, Bacillus subtilus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus mutants and Salmonella typhimurium* used for the present study were received from Bose clinical laboratory, Madurai. It was confirmed by various morphological and biochemical test (Bergye's manual of systematic bacteriology).

Fish pathogens

The bacterial fish pathogens *Aeromonas sp., Pseudomonas sp., Flexibacter sp., Yersinia sp., Flavobacterium sp.* and *Vibrio cholera* were isolated from infected fish. These strains also identified by morphological and various biochemical test (Bergye's manual of systematic bacteriology).

Antibacterial assay

Growth inhibition of pathogens by seaweeds extracts was assessed using the disc diffusion assay [16]. The concentrated crude extracted impregnated discs (100µg/ml), positive control (Ampicillin 50 µg/ml) and negative control (solvents) disc was allowed to air dry and were subsequently placed equidistantly onto the surface of the pathogen seeded Ts agar plates. The plated were kept in an inverted position and incubated at 37°C for 24 h. The growth inhibition was assessed as the diameter (in mm) of the zone of inhibited microbial growth. The experiment was carried out in triplicate.

Results and discussion

The marine environment has a great potential for the discovery of lead compounds that could be used against infectious diseases. Phyto-chemical screenings of 10 different chemical compounds (carbohydrates, proteins, fats, alkaloids, steroids, tannins, phenol, saponins, gum, and flavonoids) were tested in 5 different extracts of four seaweeds. Table 1 shows that qualitative analysis of phyto-chemicals of seaweeds powder. Thus out of (5x10=50) tests for the presence or absence of the above compounds, only 18 gave positive results and the remaining 32 gave negative results. The 18 positive results showed the presence of protein, tannin, phenol, saponins, flavonoids, steroids. Carbohydrate, fats and alkaloids and gums did not show any positive result for their presence in any of the 5 extracts tested from *Sargassum wightii*. Phenolic groups and tannins showed the maximum presence in the 5 different extracts followed by flavonoids, sapanins, steroids and proteins in 2 extracts. Among the 5 different extracts, chloroform extract showed the presence of maximum number (5) of compounds. Next to that petroleum ether and acetone extract showed 4 compounds. Butanol and methnol extracts showed 3 compounds each (Table 2).

In the present study we revealed the tannins and phenolic compound present in *S.wightii*. Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are used as healing agent in inflammation, leucorrhoea, gonorrhoea, burns piles and antidote [17]. Saponins are known to produce inhibitory effect on inflammation some of the biological properties includes antimicrobial, antifeedent and haemolytic effects [18, 19]. Flavonoids are the major groups of phenolic compound reports for their antimicrobial, antiviral and spasmolytic activity. Flavonoids ability of scavenging hydroxyl radical, superoxide anion radicals and lipid peroxy radicals highlights many of the flavonoid health promoting function in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancer as well as preventing menopausal symptoms [20, 21]. Among the 50 test, only 20 gave positive results and the remains 30 gave negative results. Phenolic tannins maximum presence in 5 different extracts followed by flavonoids in 3 extracts of *U.fasciata*. But in *C.racemosa* and *Padina gymnospora* algae contain phenol and tannin are also high in 5 different extracts followed by saponin , alkaloids and steroids (Table 2).

In this study showed that phyto-chemical compounds are varies with various solvent extractions in different algal sample. This variation of phyto-chemical compounds of our extracts might by due to location and seasons and which varied from species to species. The antibacterial activity of 5 different solvents extracts of four seaweeds against seven human bacterial pathogens was presented in Table 3. The maximum activity (16.4 mm) was recorded from the petroleum ether extracts of (*C.racemosa* against *Streptococcus mutants* and minimum (3.1mm) was observed from the acetone extracts of *Ulva faciata* against *K.pneumoniae*. In the present investigation, higher activity was recorded from the Brown alga *C.racemosa* followed by the green alga *Ulva faciata*. It has been very well established by several scientific teams that seaweeds belonging to red, brown and green algae exhibited inhibitory action against both gram positive and gram negative bacteria. Kannapiran and Nithyaandan, [22] reported that highest bacterial activity in Rhodophyta. Antibacterial activity of nine species of seaweeds belonging to brown, red and green algae revealed that red and brown seaweeds had greater antibacterial activity than the green algae [23]. But Caccamese et al., [24] has reported that the brown algal extracts showed higher activity than the extracts of red algae. In our study, the brown algae showed higher activity than the green algae (*S.wightii*). Some of the solvent extracts of algae were no inhibitory effects of some bacterial strains. This may be due to active compounds which are present or absent in plant extracts. However, some plant extracts were unable to exhibit antimicrobial activity against tested bacterial strains. Schwarz and Noble, [25] suggested that the bacterial strains may have some kind of resistant mechanisms e.g., enzymatic inactivation ,target sites, modification and decreased intracellular drug accumulation or the concentration of the compound used may not be sufficient. Brown algae show higher degree of antibacterial activity than extracts obtained from red and green algae [26] which is in coincided to the present investigation.

The five different solvent extracts prepared individually from 4 different algae belonging to two classes (Chlorophyceae and Phaeophyceae) showed various degrees of activity against fish pathogenic bacteria (Table 4). The extracts of all the algae compounds showed higher activity (86.7 %) against tested bacteria. The acetone extracts showed moderate activity towards fish pathogens. All the extracts showed 100% antimicrobial activity against *P.aeruginosa,Ulva faciat* and *P.gymnospora* showed highest (19.8 mm) antimicrobial activity against fish pathogens namely *Flexibacter sp* and *Pseudomonas sp*. It was more action against Gram negative bacteria. No inhibition could be noted for *V.cholera* by chloroform extracts of *C.racemosa* and *P.gymospora.* Johnsi. Christobel et al., [27] reported that *Sargassum wightii* showed highest antimicrobial activity against clinical pathogens namely *P.aeruginosa*, *K.pneumoniae* and *E.coli* and fish pathogens *V.alginolyticus*. In addition to that, the resistant displayed by the pathogens might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extracts.

In conclusion, the present study, it was observed that the seaweeds which were used in this work exhibited good antibacterial activity to all gram negative and positive bacteria except a few. Further study is in progress to find out the fraction of these compounds by TLC and FTIR analysis and to study the antioxidant and anti-inflammatory properties.

Table 1: Qualitative studies of phyto-chemical of selected seaweed powder

S.no	Name of compound	Name of thetest	*Sargassum wightii*	*Ulva fasciata*	*Cauterpa racemosa*	*Padina gymnospora*
1	Carbohydrate	Benedicts	+++	+++	++	+++
2	Proteins	Biuret	+++	++	++	++
3	Fats	Spot test	-	-	-	-
4	Alkaloids	Wagner's	-	-	-	-
5	Steroids	Chloroform+ Acetic acid+ H_2So_4	-	-	-	-
6	Tannin& Phenols	5%Ferric chloride	++	++	-	+

7	Saponins	Foam test	+++	+++	++	++++
8	Gum	Alcoholic precipitation	++	++++	++	++
9	Flavonoids	NaoH/Hcl	-	-	-	
10	Volatile oil	Hydrodistillation	-	-	-	-

Concentration: += 20%; ++= 40%; +++= 60%; ++++= 80%; --=Absent

Table 2: Phyto-chemicals screening of different solvent extracted seaweeds

Name of the Seaweeds	Phyto-chemicals	A	B	C	D	E
	Carbohydrates	-	-	-	-	-
	Proteins	++	-	-	+	-
Sargassum wightii	Fats	-	-	-	-	-
	Alkaloids	-	-	-	-	-
	Steroids	-	+	-	-	+
	Tannin	+	+	+	++	+
	Phenol	+	+	++	+	+
	Saponins	+++	-	++	+	-
	Gum	-	-	-	-	-
	Flavanoids	+	-	++	-	-
	Carbohydrates	+++	+	-	-	+
	Proteins	-	-	-	-	-
Ulva fasciata	Fats	-	-	-	-	-
	Alkaloids	-	-	-	-	-
	Steroids	+++	-	-	+	-
	Tannin	+	+	+	+	+
	Phenol	+	+++	+	++	+++
	Saponins	++	-	+++	-	-
	Gum	-	-	-	-	-
	Flavanoids	++	+	-	-	+
	Carbohydrates	+	-	-	+	-
	Proteins	-	-	-	-	-
Cauterpa racemosa	Fats	-	-	-	-	-
	Alkaloids	+	-	++	-	++
	Steroids	+	-	-	-	-
	Tannin	+	+	++	++	+++
	Phenol	+	+	+	+	+
	Saponins	-	++	-	++	-
	Gum	-	+	+	-	-
	Flavanoids	-	-	-	+	+
	Carbohydrates	-	-	-	+	+
	Proteins	-	-	-	-	-
Padina gymnospora	Fats	-	-	-	-	-
	Alkaloids	-	++	-	-	+
	Steroids	+	-	++	-	-
	Tannin	+	++	++	++	+
	Phenol	+++	++	++	+	+
	Saponins	-	+	+	++	-
	Gum	-	-	-	-	-
	Flavanoids	-	+	-	+	-

A-Chloroform, B-Methanol, C- Petroleum ether, D- Acetone, E- Butanol

Table 3: Zone of inhibition (mm) of some solvent extracts of selected marine algae against gram positive and gram negative bacteria

Seaweeds extracts	A	B	C	D	E	F	G
Control							
Chloromphenicol	**9.3**	**12.0**	**14.9**	**13.5**	**8.5**	**12.5**	**13.5**
Tetracycline	**13.9**	**17.2**	**16.8**	**19.6**	**14.9**	**15.6**	**7.8**
Chloroform Extracts							
S.wightii	8.2	-	11.0	10.6	10.0	9.6	10.6
U.fasciata	-	11.4	11.2	11.2	-	-	-
C.racemosa	9.1	10.2	13.3	-	9.2	10.3	12.2
P.gymnospora	12.6	9.0	12.0	8.0	10.8	9.4	-
Methanol Extracts							
S.wightii	15.0	12.6	-	9.4	11.7	11.2	9.6
U.fasciata	-	-	-	-	-	-	6.0
C.racemosa	10.2	10.8	10.5	9.1	-	11.8	12.2
P.gymnospora	14.1	-	-	12.4	14.6	-	11.4
Petroleium ether							

Extracts							
S.wightii	14.0	14.0	12.2	9.8	10.4	11.8	6.9
U.fasciata	13.4	14.1	-	-	9.8	8.1	-
C.racemosa	11.5	16.4	14.5	12.4	11.6	9.0	12.2
P.gymnospora	9.9	14.8	10.1	17.7	8.4	-	14.4
Acetone Extracts							
S.wightii	12.2	-	15.2	11.0	9.3	16.0	6.2
U.fasciata	11.5	9.1	9.6	15.2	8.1	12.5	-
C.racemosa	9.3	8.5	-	6.9	-	14.0	-
P.gymnospora	6.5	9.0	-	8.2	13.6	14.2	5.6
Butanol Extracts							
S.wightii	11.0	-	12.0	8.2	-	6.9	6.5
U.fasciata	12.3	6.5	11.2	7.6	-	8.1	4.8
C.racemosa	14.5	9.8	10.4	12.9	12.1	5.2	8.9
P.gymnospora	-	6.7	9.6	12.3	6.9	4.2	7.6

A= *S.aureus;* B= *S.mutant*; C= *B.subtilus;*D= *P.aeruginosa;*E= *K.pneumonia;* F= *E.coli*; G=*Salmonella typhimurium*

Table 4: Zone of inhibition (mm) of some solvent extracts of selected marine algae against Fish pathogens

Seaweeds extracts	**A**	**B**	**C**	**D**	**E**	**F**
Control						
Chloromphenicol	**10.5**	**12.5**	**19.0**	**14.8**	**7.5**	**12.5**
Tetracycline	**11.7**	**14.8**	**14.8**	**17.1**	**12.2**	**14.6**
Chloroform Extracts						
S.wightii	8.7	8.5	14.5	-	9.0	11.6
U.fasciata	4.2	10.4	19.8	10.2	4.9	5.6
C.racemosa	7.8	8.2	11.3	6.4	5.4	-
P.gymnospora	17.6	8.8	15.1	6.0	12.8	-
Methanol Extracts						
S.wightii	15.5	10.2	4.7	8.4	15.7	-
U.fasciata	9.4	8.9	-	5.5	5.8	5.8
C.racemosa	11.2	8.7	11.5	7.6	-	11.8
P.gymnospora	9.1	7.5	6.2	18.4	19.6	4.9
Petroleium ether Extracts						
S.wightii	10.0	8.0	7.2	6.8	-	5.2
U.fasciata	11.1	9.5	9.2	8.5	4.7	2.5
C.racemosa	14.0	12.4	10.4	-	8.6	7.2
P.gymnospora	9.4	19.8	5.5	11.8	3.8	-
Acetone Extracts						
S.wightii	10.2	12.0	5.2	10.0	5.3	14.0
U.fasciata	-	9.5	8.6	11.2	5.1	11.5
C.racemosa	-	7.9	4.8	6.8	-	9.0
P.gymnospora	8.5	5.0	-	7.9	10.6	4.2
Butanol Extracts						
S.wightii	-	7.5	-	7.2	4	11.5
U.fasciata	11.3	5.5	11.2	4.6	5.8	8.5
C.racemosa	9.2	9.5	-	10.9	10.1	5.1
P.gymnospora	5.6	4.2	8.6	8.3	6.5	7.2

A=*Aeromonas sp* ; B= *Pseudomonas sp* ;C= *Flexibacter sp.* D= *Yersinia sp.* E= *Flavobacterium sp*. F=*V.cholerae*

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