Open Access Library Journal
2018, Volume 5, e4258
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Sargassum, *Gracilaria* and *Ulva* Exhibit Positive Antimicrobial Activity against Human Pathogens

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How to cite this paper: Mishra, A.K. (2018) *Sargassum*, *Gracilaria* and *Ulva* Exhibit Positive Antimicrobial Activity against Human Pathogens. *Open Access Library Journal*, **5**: e4258.
https://doi.org/10.4236/oalib.1104258

Received: December 15, 2017
Accepted: January 8, 2018
Published: January 11, 2018

Abstract

Bacterial resistance to pharmaceutical drugs is on rise, which emphasizes the need for screening of new drugs from natural resources. Seaweeds from the marine ecosystem are important source of bioactive compounds making them one of the major subjects for screening of various pharmaceutical drugs. So here, we assessed the bacterial growth inhibitory functions of four seaweeds *Sargassum wightii*, *Gracillaria edulis*, *G. corticata* and *Ulva lactuca* of Andaman Sea and Bay of Bengal, India respectively against three pathogens *Pseudomonas aeruginosa*, *Eischeira coli* and *Staphylococcus aureus*. Solvent extraction of four seaweeds was performed using 70% methanol, ethanol and ethyl acetate. Agar well diffusion method was used to test the bioactivity of seaweeds against pathogens. *S. wightii*, *G. edulis* and *U. lactuca* were observed with better solvent extracts compared to *G. corticata*. Methanol extract of *S. wightii* was observed with the highest (29.0 ± 1.22) zone of inhibition (ZOI) and ethyl acetate extract of *U. lactuca* was observed with the lowest ZOI (5.0 ± 0.0) against *S. aureus*. Butanol extract of *S. wightii* was observed with the highest ZOI (14.0 ± 0.83) against *P. aeruginosa*, whereas *G. edulis* methanol extract and *U. lactuca* ethyl-acetate extract were observed with the lowest ZOI (6.0 ± 0.0). For *E. coli*, butanol and methanol extracts of *G. edulis* and *U. lactuca* showed the highest (12.0 ± 0.54) and the lowest (6.0 ± 0.0). Our preliminary results suggest bioactivity of *S. wightii*, *G. edulis* and *U. lactuca* showed positive results. Further biochemical characterization of *S. wightii* should be carried out for potential bioactive compounds against human pathogens. Our results suggest bioactive compounds from seaweeds can be used as pharmaceutical drugs.

Subject Areas

Ecology, Marine Biology, Microbiology

Keywords

Bioactive Compounds, Antibiotics, Human Pathogens, Indian Ocean, Solvent Extraction

1. Introduction

Antibiotics resistance is one of the biggest threats to global food security, global health and development of mankind [1]. To overcome the resistance of bacterial pathogens, continuous screening and development of potential new drugs from natural products are necessary. Natural products from marine ecosystems are diverse source of bioactive compounds due to the harsh environmental conditions in which the organisms survive [2]. In these environments, the organisms produce secondary metabolites to overcome the surrounding competition for food, habitat, to escape predation, to maintain homeostasis in the environment and to defend themselves against grazing and biofouling organisms [3] [4]. These secondary metabolites are a continuous source of bioactive compounds ranging from microalgae, coral reefs, sponges, fishes to macroalgae or seaweeds [4] [5].

The coastal zone of India is diverse and harbours various kinds of seaweeds in the intertidal region or estuarine zone of coastal ecosystems [5]. These marine macrophytes or seaweeds are multicellular algae categorised into three main groups of Chlorophyceae (Green algae), Phaeophyceae (Brown algae) and Rhodopycae (Red algae) based on their colored pigments [6] [7]. Seaweeds from all three groups are used in various industries for agar production, used in agriculture as fertilizer, food and fodder and medicines [8] [9]. These seaweeds possess various sources of secondary metabolites that possess antimicrobial properties [10]. These secondary metabolites comprise of diverse type of compounds [4], for example, polysaccharides and derived oligosaccharides like alginates, carrageenans, galactans, laminarians, fucans and ulvans [11] [12], lipids, fatty acids and sterols like phospholipids, glycolipids, carboxylic acids, fucosterol [13], phenolic compounds [14], pigments like carotenoids [15] and other compounds like lectins [16], alkaloids [17] and terpenes [18]. Presence of these various compounds makes the seaweeds valuable in pharmaceutical industries, where they have been largely screened in drug development for antibacterial, antifungal [19] [20], antiviral [21] and antitumor activities [22].

Studies on bioactivity of seaweeds in India have considered various solvent extraction procedures to test the antibacterial activity of seaweeds on human pathogens. For instance, five different species of *Gracilaria* (brown algae) of South coast of India were extracted using 10 organic solvents and five different human pathogens and only two solvents, *i.e.* isoamyl alcohol and chloroform were observed with antibacterial activity against all pathogens [23], whereas methanol extract of *G. edulis* from South coast of India showed maximum inhibitory against bacterial (*Staphylococcus aureus*, *Eischeira coli* and *Pseudomonas*

aeruginosa) and fungal pathogens [24]. Similarly, methanol extract of only *Dictyosphaeria cavernosa* (green algae) was observed with antibacterial activity against *S. aureus* out of five different seaweed species of Andaman Sea screened for antibacterial activity [25], whereas green algae of Andaman Sea, *Halimeda opuntia* ethanol extracts showed maximum antibacterial activity against *E. coli* and *S. aureus* [26]. *Ulva reticulata* n-butanol extracts were effective against *E. coli* while screened for antibacterial activity [27], whereas *Ulva lactuca* chloroform extracts were effective against *S. aureus*, *E. coli* and *P. aeruginosa* [28] [29]. However, methanol extracts of both *U. reticulata* and *U. lactuca* were effective against human pathogens [30]. In case of *Sargassum wightii* methanol extracts were effective against *S. aureus* [31] and against *E. coli*, and *P. aeruginosa* [32], whereas ethyl acetate extracts of *S. wightii* were only effective against *Bacillus subtilis* [33].

However, considering the growing need for new source of bioactive compounds to fight microbial resistance, here in our research we are screening a combination of red, green and brown algae against human pathogens via solvent extraction procedures. Solvent extracts of *Sargassum wightii* (Brown algae) and *Gracilaria edulis* (Red algae) of Andaman Sea and *G. corticata* (Red algae) and *Ulva lactuca* (Green algae) of South-east coast of Tamilnadu, India against three human pathogens (*Pseudomonas aureus*, *Eischeira coli* and *Staphylococcus aeruginosa*) to understand the bioactivity of these seaweeds against similar pathogens.

2. Methods

2.1. Seaweed Collection

Seaweeds, *Sargassum wightii* and *Gracillaria edulis* samples were collected from the islands of Andaman and Nicobar in Andaman Sea, India. Samples of *Ulva lactuca* and *Gracillaria corticata* were collected from the southeast coast of Tamilnadu in Bay of Bengal, India. Identification of seaweeds was done at the Department of Ocean Studies and Marine Biology, Portblair, Andaman and Nicobar Islands. After collection all the seaweed samples were washed with distilled water thrice to remove the epiphytes and debris attached to the blades and dried in shade. Sun drying or hot air drying was avoided to save the volatile compounds that escape at higher than room temperature.

2.2. Solvent Extraction

Dried seaweed blades of approximately 2 gm were crushed in a mortar and were place in culture bottles along with 25 ml of solvents for extraction. Three solvents were used, *i.e.* methanol, butanol and ethyl acetate for each seaweed sample. These culture bottles were covered with aluminium foils and were kept in normal room temperature in a shaker (50 - 70 rpm) for two days for the extraction of bioactive compounds by the solvent. Distillation process was used for solvent extraction.

2.3. Pathogenic Organisms and Biochemical Characterization

The pathogenic organisms *Pseudomonas aureus*, *Eischeira coli* and *Staphylococcus aeruginosa* were selected from clinical samples. The collected samples were incubated in nutrient broth and incubated for 24 hours at 37˚C. After the initial incubation the organisms were cultured again in nutrient agar medium and incubated for another 24 hours at 37˚C to get isolated pure cultures. Gram staining was performed for these isolates to confirm, if they are gram positive or negative. The morphologically identified organisms were then grown on selective media such as Eosin Methylene Blue (EMB) agar, starch agar, Mannitol salt agar and blood agar. To confirm the specificity of each bacteria used, the selectively grown isolates were characterized by various biochemical tests. After confirmation through various biochemical tests the microbes were isolated and cultured in nutrient agar plates for maintaining pure culture and used for antimicrobial activity tests.

2.4. Determination of Antimicrobial Activity

Antimicrobial activity of seaweed samples was determined by agar diffusion method. Five identical colonies of *E. coli*, *S. aureus* and *P. aeruginosa* were lifted with sterile loop from each pure culture agar plates and transferred into a sterile tube containing 5 ml of nutrient broth. These tubes were incubated at 37˚C for 24 hours. Then Muller Hilton (MH) agar was prepared sterilized and was poured on petri dishes and cooled. Into these sterile petri dishes with MH agar medium fresh cultures of microbes (0.1 ml) were inoculated from nutrient broth. Each inoculated petri dishes were swirled to distribute the medium homogeneously and allowed to dry for 15 - 20 minutes. Wells (n = 5) of 7 mm were made into previously seeded MH agar plates. Each well was filled with 50 µl each plant extract. Same petridishes were used as controls, where instead of plant extract 75% ethanol was used. Petri dishes were kept in room temperature around 1 hour for the seaweed extract to diffuse and then were incubated at 37˚C for 24 hours. Subsequently, the dishes were examined for bacterial growth inhibition. The diameter of cleared zones was measured in millimetre (mm). Transparent clear zones were considered to have bacteriostatic activity. All values are expressed as mean ± standard deviation (SD). All values were tested for normality and standard deviation.

3. Results

The three solvents used for seaweed extraction showed a significant variation in their extraction capacities. The percentage of extraction for each seaweed was different for each solvent with highest extractions of seaweed being observed in ethyl-acetate followed by butanol and methanol (Table 1). In methanol and ethyl-acetate *S. wightii* showed the highest extraction, whereas in butanol *U. lactuca* extraction was highest. In all three solvents, *G. corticata* showed the lowest extraction (Table 1).

Both *E. coli* and *P. aeruginosa* were gram negative with greenish colour where *S. aureus* was gram positive with golden yellow colour as observed from the colonies in selective growth media and gram staining procedures (Table 2). The results of various biochemical tests for the selected pathogens are presented in Table 3. Both *P. aeruginosa* and *E. coli* were showed positive results for Indole production, oxidase and nitrate reduction test, whereas *E. coli* showed positive results for catalse activity. Similarly, *S. aureus* showed positive results for Voges-Proskauer, gelatine, catalase and nitrate reduction test (Table 3).

Zones of inhibition (ZOI) that determine seaweeds antimicrobial activity were significantly different between for the extracted solvent and the pathogens used (Table 4). 4-fold higher ZOI was observed for *S. wightii* methanol extract (29.0 ± 1.22) than ethyl-acetate extract (6.0 ± 0.44) against *S. aureus* (Table 4). In case of *G. edulis* both methanol and butanol extract were observed with similar ZOI range for all the three pathogens, exception was ethyl-acetate extract with only observable ZOI for *P. aeruginosa*. In *G. corticata* there were no observed ZOI for methanol extract, whereas butanol extract against *P. aeruginosa* was observed with highest ZOI. In *U. lactuca* 2-fold higher ZOI was observed for butanol extract against *P. aeruginosa* and *E. coli* than ethyl-acetate extract against *S. aureus* (Table 4). Overall the highest and lowest ZOI were observed for gram positive *S. aureus* in our results.

Table 1. Weight loss (n = 5, Mean ± SD) of four seaweeds during solvent extraction in Methanol (M), Butanol (B) and Ethyl acetate (EA).

Seaweed	Solvent	Initial weight (gm)	Final weight (gm)	Weight loss (gm)
S. wightii	M	2.29 ± 0.31	1.95 ± 0.02	0.33 ± 0.05
	B	2.28 ± 0.04	1.87 ± 0.01	0.40 ± 0.05
	EA	2.25 ± 0.01	1.64 ± 0.02	0.61 ± 0.03
G. edulis	M	2.40 ± 0.04	1.97 ± 0.01	0.42 ± 0.04
	B	2.14 ± 0.02	1.62 ± 0.02	0.52 ± 0.02
	EA	2.20 ± 0.03	1.61 ± 0.01	0.59 ± 0.05
G. corticata	M	2.29 ± 0.03	1.21 ± 0.02	0.07 ± 0.03
	B	2.40 ± 0.04	2.35 ± 0.04	0.04 ± 0.00
	EA	2.21 ± 0.02	2.18 ± 0.01	0.03 ± 0.01
U. lactuca	M	2.40 ± 0.04	2.18 ± 0.04	0.21 ± 0.23
	B	2.28 ± 0.04	1.77 ± 0.03	0.50 ± 0.04
	EA	2.40 ± 0.04	2.18 ± 0.04	0.21 ± 0.02

Table 2. Morphological response of the pathogens during growth in selective media and Gram staining.

Media	Observation	Pathogens	Staining results
EMB agar	Greenish metallic sheen	*E. coli*	*Gm − ve*
Mannitol salt agar	Golden yellow colonies	*S. aureus*	*Gm + ve*
Centrimide agar	Green tinch	*P. aeruginosa*	*Gm − ve*

Table 3. Results of various biochemical tests of *P. aeruginosa*, *S. aureus* and *E. coli*. Positive (+) and negative (−) represent positive and negative response for biochemical tests for each pathogen.

Test	*P. aeruginosa*	*S. aureus*	*E. coli*
Indole	+	−	+
Methyl red	−	−	−
Voges-Proskauer	−	+	−
Gelatine	−	+	−
Catalase	−	+	+
Oxidase	+	−	+
Nitrate	+	+	+
Starch	−	−	−
Citrate	−	−	−

Table 4. Zone of inhibition (n = 5, Mean ± SD) observed for each seaweed species with solvent extraction in Methanol (M), Butanol (B) and Ethyl acetate (EA). No zone of inhibition is represented by (−).

Seaweed	Solvent	Zone of inhibition (mm)		
		P. aeruginosa	*S. aureus*	*E. coli*
S. wightii	M	7.0 ± 0.54	29.0 ± 1.22	-
	B	14.0 ± 0.83	10.0 ± 0.54	11.0 ± 0.54
	EA	9.0 ± 0.70	6.0 ± 0.44	7.0 ± 0.44
G. edulis	M	6.0 ± 0.54	6.0 ± 0.54	6.0 ± 0.54
	B	12.0 ± 0.57	13.0 ± 0.57	12.0 ± 0.54
	EA	11.0 ± 0.83	-	-
G. corticata	M	-	-	-
	B	10.0 ± 0.44	9.0 ± 0.54	9.0 ± 0.0
	EA	7.0 ± 0.44	7.0 ± 0.54	7.0 ± 0.0
U. lactuca	M	8.0 ± 0.44	6.0 ± 0.0	6.0 ± 0.54
	B	12.0 ± 0.54	11.0 ± 0.83	12.0 ± 0.83
	EA	6.0 ± 0.0	5.0 ± 0.0	8.0 ± 0.0

4. Discussion

Antimicrobial activity of *S. wightii*, *G. edulis*, *G. corticata* and *U. lactuca* from Andaman Sea and Bay of Bengal, India was screened against *S. aureus*, *P. aeruginosa* and *E. coli* through solvent extraction procedures in our studies. The extraction of seaweed bioactive compounds through solvent extracts was different due to the various nutritive and antioxidant contents of the seaweed, similar difference in extractions has been observed previously for solvent extracts of other seaweeds [34].

Methanol extract of *S. wightii* showed the highest ZOI for *S. aureus* though the weight loss in the extraction was less than butanol and ethyl acetate (**Table**

1). This suggests that methanol is a better solvent for consistent extraction of bioactive compounds from brown seaweeds, which was observed previously for plants [35]. The capacity of methanol for better extraction is due to the enhancement of methanol soluble bioactive components of *S. wightii* like alkaloids, steroids, flavonoids, essential oils and biterpenoids resulting in higher number of bioactive compounds extracted from the macroalgae [36].

We observed red, green and brown seaweed possessing different levels of bioactivity when extracted through various solvents. This indicates seaweeds biochemical composition and growth stage play a major role in producing bioactive compounds that are extractable through various solvents [37]. Secondly the various solvents used are different in their chemical composition that also affects the extraction of bioactive compounds. Thirdly, the bioassay methods, geographical distribution of seaweeds and seasonal production of bioactive compounds also contribute to the efficient bioactive property of seaweeds [38].

Methanol extracts of seaweed showed the highest ZOI for bacterial pathogens, suggesting methanol as one of the better solvents than butanol and ethyl-acetate, which has been previously observed for methanol [39] [40] [41] [42]. The highest ZOI against *S. aureus* (Gram positive bacteria) in our results for methanol extract of *S. wightii* coincides with previous cases where methanol extracts provided the highest ZOI, suggesting methanol extracts of seaweeds are efficiently bioactive against Gram-positive bacteria than Gram-negative bacterial species [24] [41] [42].

In our results, ZOI formed by *S. wightii* methanol extracts against *S. aureus* was the highest, which was 2.9-fold higher than previously observed for *S. wightii* methanol extract from Mandapam [31] and agreed that methanol extract for *S. wightii* against *S. aureus* was better than other solvents [42]. Though, *S. wightii* butanol and ethyl acetate extracts showed considerable bioactivity against both *S. aureus*, *P. aeruginosa* and *E. coli* in our results, *S. wightii* methanol extracts showed no bioactivity against *E. coli*, which agreed with the findings for *Sargassum vulgare* with no activity [44] and disagreed with observations for *S. wightii* [45]. This difference in bioactivity against *E. coli* for *Sargassum* species can be due to the different antibacterial compounds which these species harbour and their interaction with pathogens [33].

Methanol extraction of *G. edulis* in our studies formed 6-fold higher ZOI against *S. aureus* and *P. aeruginosa* and 3-fold lower ZOI against *S. aureus* than previously observed for *G. edulis* from Tamilnadu, India [23] [24], whereas methanol extracts of *G. corticata* showed no ZOI against *S. aureus* and *P. aeruginosa* in our results. Lower or no activity of *Gracilaria* species in our results can be due to the lower biomass (2 mg) used for solvent extraction, as previous studies on *G. corticata* showed considerable antibacterial activity against *S. aureus* and *P. aeruginosa* in methanol extracts when 4 - 5 mg of dried biomass is used [24] [44].

Methanol and ethyl acetate extracts of *U. lactuca* formed 2-fold lower ZOI

against *S. aureus* in our studies, whereas for *P. aeruginosa*, the ZOI was not very different and for *E. coli* ZOI was almost 2-fold lower than methanol extract and similar with ethyl acetate extract, than results obtained for *U. lactuca* from South coast of India [30].

The differences in antimicrobial activity of various seaweeds analysed in our research were different, which is a result of various factors such as herbivory, light depth, nutrients and the growing environmental conditions [45]. However, our results showed that seaweeds growing in oligotrophic trophic waters of Andaman Sea have higher bioactivity than the seaweeds growing in nutrient rich waters of the South coast of India. This phenomenon can be due to nutrient limitation in oligotrophic waters for seaweeds as a result they need to harbour all nutrients in their blades, attracting higher microbial organisms, thus high bioactivity. Secondly the various compounds seaweeds harbour like steroids and phenols also determines their antimicrobial activity, which helps in inhibiting microbial growth by acting on the bacterial cell wall [46] [47].

Our results suggest Gram-positive bacteria (*S. aureus*) were more susceptible to seaweed extracts than Gram-negative bacteria (*E. coli and P. aeruginosa*). Similar results have been observed for seaweed extracts against Gram-positive bacteria elsewhere [41] [48]. This difference in response to various seaweed extracts between Gram positive and negative are due to their cell wall structure and chemical composition [42] [49], where Gram negative bacterial species have a thicker outer membrane and murine layer acting as a barrier to many environmental substances and inhibitors and Gram-positive bacteria lacking these features are susceptible to bioactive compounds [50] [51].

This study analysed the bioactive potential of four seaweed species against three common human pathogens through solvent extraction method and observed that *S. wightii* was the most effective seaweed against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* and *P. aeruginosa* followed by *G. edulis* and *U. lactuca*, whereas *G. corticata* was the least effective against both Gram positive and negative bacteria. The antimicrobial property exhibited by these seaweeds suggest they have a great potential to be screened for various antibacterial compounds depending on the biochemical composition of red, brown and green seaweeds. This preliminary screening suggests further biochemical characterization of vast source of seaweed secondary metabolities are necessary for discovering new bioactive compounds for various antibacterial drugs to fight against the antibiotics resistance of the 21st century and further.

Acknowledgements

This work was carried out as summer training internship at San Genomics Research Laboratory, Bangalore, India. I am thankful to Dr. M. P. Prasad for providing laboratory facilities for carrying out the research. I am grateful to Nitya Shree and Sasi Kumar for the helping hands in laboratory works. I am thankful to Anchal Paramguru for her initial comments on the manuscript.

Conflict of Interest

The author declares there is no conflict of interest between any organizations for funding or any other reasons.

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