



Seasonal Variation in Antibacterial Activity of Seaweed *Hypnea Valentiae* and Its Epiphytic Bacteria

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ABSTRACT

The seaweed *Hypnea valentiae* was extracted with solvents hexane, acetone and methanol. In the preliminary screening, hexane extract showed wide spectrum activity against ten human bacterial pathogens. The maximum level inhibition was observed against *Escherichia coli*, *Vibrio parahaemolyticus* and *Micrococcus luteus* and minimum against *Vibrio cholerae*. The assessment of seasonal variation in antibacterial activity with hexane extract showed higher activity during postmonsoon season followed by summer, premonsoon and monsoon season. The epiphytic bacterial density on surface of *Hypnea valentiae* was 25×10^3 CFU/cm². Ten epiphytic bacteria (HV1- HV10) was isolated and the strain HV10 showed higher activity which was identified based on the morphologically and biochemical character as *Alcaligenes* sp. It was further cultured and the culture supernatant was extracted with hexane, n-butanol and water. The n-butanol extract showed higher activity against *Shigella flexneri* and minimum against *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus epidermis*. The investigation has proved that seaweed *Hypnea valentiae* and its epiphytic bacterial strain could be the potential source of bioactive compounds of biomedical importance.

Keywords: *Hypnea valentiae*, Antibacterial activity, Epiphytic bacteria and Solvents extraction

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INTRODUCTION

The marine macroalgae occupy an important place as a source of biomedical compounds^{1, 2}. About 2400 natural products have been isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae³. The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macroalgae for its synthesis of bioactive secondary metabolites^{4, 5}. The compounds derived from macroalgae are reported to have broad range of biological activities such as antibacterial^{6, 7, 8} anticoagulant⁹ and antifouling activity^{10, 11}. Macro algae are considered as very important source of bioactive natural products and many species of red, green and brown macro and micro algae have been screened to see if they contain substances with antibacterial activity¹².

These epibiotic bacterial secondary metabolites have the potential for applications in pharmaceutical or industrial field and have provided an opportunity to meet the growing demand for new compounds of natural origin. The marine microorganisms are of considerable current interest as a new promising source of bioactive substances. Marine microorganisms, especially bacteria, often are living in close association with higher organisms. These associations have attracted much interest during recent years, because of the production of substantial number of valuable bioactive compounds¹³. Marine bacteria often produce anticancer and antibacterial substances as a means of maintaining relationships between epiphytic micro-environments, inhibiting competing organisms and microbial pathogens¹⁴.

Bacterial infection causes high rate of mortality in human population and aquaculture organisms¹⁵. For example, *Bacillus cereus* is responsible for causing food borne diseases¹⁶. *Enterococcus faecalis* is the causative agent of inflammatory bowel disease¹⁷. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella* sp. causes diarrhoea and typhoid fever¹⁸. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness¹⁵. Seaweeds have been used in traditional medicine for a long time¹⁹. The present investigation was carried out to assess the seasonal variation (premonsoon, monsoon, postmonsoon and summer) in antibacterial activity from seaweed *Hypnea valentiae* and its epiphytic bacteria against ten human bacterial pathogens.

MATERIAL AND METHODS

Sample Collection

The red algae *Hypnea valentiae* (J.V. Lamouroux, 1813), was collected from the intertidal region

of Tuticorin coast, (Lat 08°45'; Long 78°12'E) Gulf of Mannar, South east coast of India. Algal samples were washed with sterile sea water to remove extraneous matter and necrotic parts. Seaweed were collected for each season during 2012-2013 (Premonsoon (July-September 2012); Monsoon (October- December 2012); Postmonsoon (January-March 2013) and summer (April-June 2013).

Preparation of Seaweed Extract

Then seaweed was shade dried in dark (in order to avoid photolysis and thermal degradation of the metabolites) at room temperatures until steady dry weight was obtained²⁰. The shade dried, seaweed was cut into small pieces and powdered in a mixer grinder. The powdered seaweed samples were separately extracted with hexane, acetone and methanol solvents thrice by soaking overnight at -18⁰ C. The extracts were filtered through Whatman no.1 filter paper and the residues were evaporated and concentrated²¹. The crude extracts were tested for antibacterial activity against human pathogenic bacteria.

Test Organisms

The hexane, acetone and methanol crude extracts of seaweed were screened against 10 human pathogenic bacteria such as *Escherichia coli*, *Shigella flexneri*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Streptococcus epidermidis* and *Micrococcus luteus*, obtained from Christian Medical College (CMC), Vellore.

Antibacterial Assay

The antibacterial activity was carried out by following the standard agar disc diffusion method using Mueller Hinton agar²². The 10 human pathogenic bacteria were inoculated in nutrient broth and incubated for 24 hrs prior to their use in the antibacterial assay. In the preliminary assay, 250 µg of crude hexane, acetone and methanol extracts were used. For seasonal variation study, different concentrations of the crude hexane extract (50 µg, 75 µg, 100 µg, 125 µg and 150 µg/disc), which showed higher activity in the preliminary assay, were used. The extracts were loaded on a sterile Whatman No.1 filter paper discs of 6 mm diameter, the disc was allowed to dry and then placed aseptically over the plates of Mueller Hinton agar swabbed with test pathogens and was incubated at room temperature for 24 hrs. The control plates with disc inoculated with solvent alone were also maintained separately. The zone of inhibition around the discs after the incubation was measured (mm) from the edge of the disc to the end of clear zone.

Isolation of epiphytic bacteria from *Hypnea valentiae*

The surface associated seaweed epiphytic bacteria were isolated by the method Murugan and

Santhana²². A sterile plastic film with 0.5 cm² hole was placed on the surface of the seaweed and the area within the hole was swabbed with a sterile cotton swab. The swabs were then transferred to sterile glass tubes containing sterile seawater which was then serially diluted and pour plated into zobell marine agar plates. The plates were incubated at room temperature for 24 hrs. The bacterial colonies obtained were counted and expressed as colony forming units (CFU) per cm². The sampling was done in triplicate and the average has been taken. Ten morphologically different strains of epiphytic bacteria were selected and isolated by repeated streaking. The isolated epiphytic bacterial strains were stored in zobell marine agar slants at 4°C.

Preliminary screening of culture supernatants of epiphytic bacteria for antibacterial activity

Antibacterial assay was used as a preliminary screening to isolate antagonistic bacteria against human bacterial pathogens. The standard agar disc diffusion method was used to assess the antibacterial activity of bacterial supernatants. The selected bacterial strains were inoculated into 25 ml of zobell marine broth and incubated on a shaker at 120 rpm for 48 hrs. At the end of incubation period, the fermentation medium was centrifuged at 5000 rpm for 15 minutes to separate the cell mass. The collected supernatant was concentrated. The residue was diluted with 1 ml of distilled water and then 100 µl of the supernatant was loaded on to the respective 5 mm wells of the Mueller-hinton agar medium seeded with test pathogens. The plates were incubated at room temperature for 24 hrs and then observed. The zone of inhibition around the well was measured from the end of the well to the end of the clear zone. Based on the activity in supernatant, active strain was further selected for extraction of secondary metabolites using different solvents. The active strain HV10 was identified based on the morphologically and biochemical characters.

Solvent extraction of bacterial metabolites

The extraction of bacterial metabolites was carried out by following the method of Zheng *et al*²³. The one potent active strain (HV10) observed in preliminary screening was inoculated into 100 ml zobell marine broth and incubated in a shaker at 120 rpm for 48 hrs. After the incubation period, the broth culture was centrifuged at 5000 rpm for 15 min. The supernatant was extracted twice with equal volume of hexane, butanol and water separately. The solvent phases were then separated using separating funnel and concentrated by evaporation. The concentrated crude extracts were dissolved in 1 ml of respective solvents and then impregnated at 100 µg/disc concentration onto sterile Whatman no. 1 discs of 6 mm diameter. The antibacterial activity was assayed by following the disc diffusion assay²². The solvents alone in the disc were used as

control. The inhibition zone was measured (millimeter) from the border of the disc to edge of the clear zone.

RESULT AND DISCUSSION

Preliminary screening for antibacterial activity

In the preliminary screening, the hexane, acetone and methanol extract of seaweed *Hypnea valentiae* showed wide spectrum activity against the ten human bacterial pathogens (Table 1). Maximum antibacterial activity was observed in the hexane extract, the supreme level of inhibition was found against *Escherichia coli*, *Vibrio parahaemolyticus* and *Micrococcus luteus* and least against *Vibrio cholera*. The extracts of solvents acetone and methanol showed moderate level activity. In general, the maximum level of inhibition was recorded against *Escherichia coli* in hexane, acetone and methanol extracts.

Table 1: Antibacterial activity of crude extracts from *Hypnea valentiae*

S. No	Human bacterial pathogens	Zone of inhibition (mm)		
		Crude extracts (250 µg/disc)		
		Hexane	Acetone	Methanol
1.	<i>Escherichia coli</i>	6	4	5
2.	<i>Shigella flexneri</i>	3	-	-
3.	<i>Bacillus subtilis</i>	4	5	3
4.	<i>Salmonella typhi</i>	4	2	-
5.	<i>Staphylococcus aureus</i>	5	4	3
6.	<i>Vibrio cholera</i>	2	3	4
7.	<i>Pseudomonas aeruginosa</i>	4	-	-
8.	<i>Vibrio parahaemolyticus</i>	6	3	4
9.	<i>Streptococcus epidermis</i>	5	-	3
10.	<i>Micrococcus luteus</i>	6	-	3

(- No activity)

Seasonal variation in antibacterial activity

The hexane extract, which showed wide spectrum activity, was selected for seasonal variation studies and various concentrations of the extract were used to assess the effectiveness of production of antibacterial compounds. Invariably seaweed collected at all the four seasons showed antibacterial activity, the higher antibacterial activity was observed in post-monsoon season followed by summer, pre-monsoon and lower in monsoon.

During premonsoon, the crude hexane extracts of *Hypnea valentiae* showed concentration dependent activity against the bacterial pathogens. The extract of 150 µg /disc concentration inhibited 70% pathogens. The maximum activity was observed against *E. coli*. The activity of extracts at other concentrations such as 125 µg /disc and 100 µg /disc were 40% and 20%

respectively. The 50 and 75 µg concentrated extracts did not show any activity against the pathogenic bacteria (Table 2).

Table 2: Antibacterial activity of *Hypnea valentiae* during premonsoon season (2009)

S. No.	Human bacterial pathogens	Zone of inhibition (mm)				
		Crude hexane extract				
		50 µg	75 µg	100 µg	125 µg	150 µg
1	<i>Escherichia coli</i>	-	-	1	1	3
2	<i>Shigella flexneri</i>	-	-	-	-	1
3	<i>Bacillus subtilis</i>	-	-	-	-	1
4	<i>Salmonella typhi</i>	-	-	-	1	-
5	<i>Staphylococcus aureus</i>	-	-	-	-	1
6	<i>Vibrio cholera</i>	-	-	-	-	-
7	<i>Pseudomonas aeruginosa</i>	-	-	-	1	-
8	<i>Vibrio parahaemolyticus</i>	-	-	-	-	1
9	<i>Streptococcus epidermis</i>	-	-	-	-	1
10	<i>Micrococcus luteus</i>	-	-	1	1	1

(- No activity)

During monsoon season, the hexane extract at 150 µg/ disc inhibited 60% bacterial pathogens. The maximum activity was observed against *Shigella flexneri*. The lower extract concentrations of 125 µg/ disc and 100 µg/ disc showed 20% and 10% activity respectively. Other concentrations did not show any antibacterial activity against the pathogens (Table 3).

Table 3: Antibacterial activity of *Hypnea valentiae* during monsoon season (2009)

S. No.	Human bacterial pathogens	Zone of inhibition (mm)				
		Crude hexane extract				
		50 µg	75 µg	100 µg	125 µg	150 µg
1	<i>Escherichia coli</i>	-	-	-	-	-
2	<i>Shigella flexneri</i>	-	-	1	1	2
3	<i>Bacillus subtilis</i>	-	-	-	-	1
4	<i>Salmonella typhi</i>	-	-	-	-	-
5	<i>Staphylococcus aureus</i>	-	-	-	-	1
6	<i>Vibrio cholera</i>	-	-	-	1	1
7	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-
8	<i>Vibrio parahaemolyticus</i>	-	-	-	-	1
9	<i>Streptococcus epidermis</i>	-	-	-	-	-
10	<i>Micrococcus luteus</i>	-	-	-	-	1

(- No activity)

During post monsoon season, the hexane extract showed maximum activity at 150 µg/disc concentration. The maximum zone was observed against *Vibrio parahaemolyticus* and minimum activity against *Vibrio cholerae*. Other concentrations such as 125 µg, 100 µg and 75 µg/ disc showed 90%, 70% and 50% activity against pathogens respectively. In 50 µg concentration, 30%

activity was observed against bacterial pathogens (Table 4).

Table 4: Antibacterial activity of *Hypnea valentiae* during post-monsoon season (2010)

S. No.	Human bacterial pathogens	Zone of inhibition (mm)				
		Crude hexane extract				
		50	75	100	125	150
		µg	µg	µg	µg	µg
1	<i>Escherichia coli</i>	1	1	2	3	4
2	<i>Shigella flexneri</i>	-	-	1	1	3
3	<i>Bacillus subtilis</i>	-	1	1	2	2
4	<i>Salmonella typhi</i>	1	1	1	2	4
5	<i>Staphylococcus aureus</i>	-	-	-	1	3
6	<i>Vibrio cholera</i>	-	-	-	-	1
7	<i>Pseudomonas aeruginosa</i>	-	-	1	1	3
8	<i>Vibrio parahaemolyticus</i>	1	1	2	3	5
9	<i>Streptococcus epidermis</i>	-	-	-	2	2
10	<i>Micrococcus luteus</i>	-	1	2	3	4

(- No activity)

During summer season, the hexane extract exhibited 90% activity against bacterial pathogens at 150 µg/disc concentration. The maximum inhibition zone was observed against *Salmonella typhi*. The 125 µg and 100 µg concentrations showed 70% and 60% activity against pathogens respectively. The lower concentrations of 75 µg and 50 µg showed activity of 10% and 30% respectively (Table 5).

Table 5: Antibacterial activity of *Hypnea valentiae* during summer season (2010)

S. No.	Human bacterial pathogens	Zone of inhibition (mm)				
		Crude hexane extract				
		50	75	100	125	150
		µg	µg	µg	µg	µg
1	<i>Escherichia coli</i>	-	1	2	2	3
2	<i>Shigella flexneri</i>	-	-	1	1	2
3	<i>Bacillus subtilis</i>	-	-	-	-	-
4	<i>Salmonella typhi</i>	-	1	1	2	4
5	<i>Staphylococcus aureus</i>	-	-	-	1	1
6	<i>Vibrio cholera</i>	-	-	-	-	1
7	<i>Pseudomonas aeruginosa</i>	-	-	1	1	3
8	<i>Vibrio parahaemolyticus</i>	1	-	1	2	2
9	<i>Streptococcus epidermis</i>	-	-	-	-	1
10	<i>Micrococcus luteus</i>	-	1	1	2	3

Epiphytic bacterial density

In this study, the epiphytic bacteria load on the surface of *Hypnea valentiae* was 25×10^3

CFU/cm². Only 10 morphologically different epiphytic bacterial strains were selected and screened for antibacterial activity against human bacterial pathogens. The isolated ten epiphytic bacterial strains were given designated codes from HV1 to HV10.

Antibacterial activity of culture supernatants of epiphytic bacteria

The preliminary screening indicated moderate activity in supernatants of three strains (HV3, HV7 and HV10). The supernatant of strain HV10 showed wide spectrum activity, it inhibited the growth of all ten human pathogens. The maximum inhibition zone was observed against *Micrococcus luteus* and minimum zone against *Bacillus subtilis* and *Vibrio cholerae*. The HV7 strain showed activity against the five pathogens and the maximum 3 mm zone was recorded against *Micrococcus luteus*. The strain HV3 showed low spectrum activity against two bacterial pathogens. Other strains did not inhibit the growth of human pathogens considerably (Table 6). The strain HV10 of marine algae *Hypnea valentiae* was further selected for solvent extraction. The active strain *Alcaligenes* sp (HV10) was identified based on the morphologically and biochemical characters (Table 7).

Table 6: Antibacterial activity of supernatants of the epiphytic bacteria from *Hypnea valentiae*

S. No.	Isolated Epiphytic bacterial strains	Human bacterial pathogens									
		<i>Escherichia coli</i>	<i>Shigella flexneri</i>	<i>Bacillus subtilis</i>	<i>Salmonell typhi</i>	<i>Staphylococcus aureus</i>	<i>Vibrio cholera</i>	<i>Pseudomonas aeruginosa</i>	<i>Vibrio parahaemolyticus</i>	<i>Streptococcus epidermis</i>	<i>Micrococcus luteus</i>
Zone of inhibition (mm) (100 µl/disc)											
1	HV1	-	-	-	-	-	-	-	-	-	-
2	HV2	-	-	-	-	-	-	-	-	-	-
3	HV3	2	-	-	-	-	1	-	-	-	-
4	HV4	-	-	-	-	-	-	-	-	-	-
5	HV5	-	-	-	-	-	-	-	-	-	-
6	HV6	-	-	-	-	-	-	-	-	-	-
7	HV7	-	1	1	-	1	-	2	-	-	3
8	HV8	-	-	-	-	-	-	-	-	-	-
9	HV9	-	-	-	-	-	-	-	-	-	-
10	HV10	4	3	2	3	4	2	3	5	4	6

(- No activity)

Table 7: Biochemical characteristics of the *Alcaligenes sp.*

Biochemical tests	Results
Gram's staining	Gram negative
Motility	+
Carbohydrate fermentation test	
a. D-glucose	+
b. Mannitol	+
c. Lactose	-
d. Sucrose	+
Indole Production	-
Methyl red test	+
Voges – Proskauer test	-
Citrate utilization test	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Urease test	+
Catalase test	+
Oxidase	+
Nitrate utilization test	-

(+ Positive Results, - Negative Results)

Table 8: Antibacterial activity of solvent extracts of culture supernatant of *Alcaligenes sp* (HV10)

Sl. No.	Human pathogenic bacteria	Zone of inhibition (mm)		
		Solvent extracts (100 µg/disc)		
		Hexane	n-Butanol	Water
1	<i>Escherichia coli</i>	-	2	1
2	<i>Shigella flexneri</i>	1	8	3
3	<i>Bacillus subtilis</i>	-	3	2
4	<i>Salmonella typhi</i>	1	4	1
5	<i>Staphylococcus aureus</i>	-	3	1
6	<i>Vibrio cholera</i>	-	5	2
7	<i>Pseudomonas aeruginosa</i>	-	2	1
8	<i>Vibrio parahaemolyticus</i>	1	6	2
9	<i>Streptococcus epidermis</i>	-	2	1
10	<i>Micrococcus luteus</i>	-	4	2

(- No activity)

Antibacterial activity of solvent extracts of culture supernatant of HV10 strain

The *Alcaligenes sp* (HV10) strain, which showed prominent activity in preliminary screening method, was subjected to secondary screening through solvent extraction of its supernatants against ten human pathogenic bacteria. The n-butanol extracts showed maximum level of inhibition against *Shigella flexneri* and minimum against *Escherichia coli*, *Pseudomonas*

aeruginosa and *Streptococcus epidermis*. The hexane and water extracts showed moderate level activity. In general, the hexane, n-butanol and water showed maximum inhibition against *Shigella flexneri* (Table 8).

Marine organisms are rich sources of structurally unique and biologically active metabolites²⁴. Seaweeds are considered as a potential source of bioactive compounds as they produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities like cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities^{25,26}. These compounds with different ecological role could be utilized as good bioactive compounds against disease resistance human pathogenic bacteria.

In the present study, *Hypnea valentiae* showed the presence of antibacterial activity against human bacterial pathogens. The activity was high in hexane extract followed by acetone and methanol extracts indicating that the seaweed might possess multiple compounds. The hexane extract, showed maximum activity against all ten bacterial pathogens especially against *Escherichia coli*. The present observation coincided with Mageid *et al*²⁷ reported that the hexane extracts of *Asparagopsis taxiformis* (red algae) showed good activity against human pathogens with highest activity against *Escherichia coli*. The observation of activity could be attributed to the production of biogenic compounds such as indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons which were found to be prevalent in red algae^{5,28,29}. But, the higher activity in hexane extract was in contrast to the Manilal *et al*^{7,30} reported that the methanol extracts of red algae *Asparagopsis taxiformis*, *Laurencia ceylanica*, *Laurencia brandenii*, *Hypnea valentiae* and *Falkenbergia hillebrandii* showed broadest and highest antimicrobial activity when compared to other solvents.

Marine bacteria are living in close association with higher organisms. These associations attracted much interest for the production of some bioactive compounds. They are surviving on the surfaces of marine algae and live in a highly competitive environment where space and nutrients are limited. Such bacteria living on the surfaces of marine organisms have been documented to be dominated by antibiotic producing bacteria compared to that of some free-living bacteria isolated from a few other marine environments^{31,23}.

In this study, the average density of surface associated bacteria on *Hypnea valentiae* was 25×10^3 CFU/cm². The present result of epiphytic bacterial population was very high when compared to the epibacterial density of 28×10^2 CFU/cm² in the seaweed *Gracilaria edulis*³², 189×10^2 CFU/cm² in *Halimeda tuna*³³, 19×10^1 CFU/cm² in *Dictyota dichotoma* and 15×10^1 CFU/cm² in *Cheatomorpha linoides*³⁴, similarly Beleneva and Zhukova³⁵ reported that surface associated

bacterial density on surface of *Camphylaeophora hyphaeoides* was 1.37×10^5 CFU/g and on *Gracilaria verrucosa* was 1.71×10^5 CFU/g. The present observation of associated bacteria on the surface of the seaweed may be attributed to the release of large amount of organic carbon into the surrounding environment³⁶ and thereby providing a nutrient rich habitat for bacteria. In several algal species, a specific vitamin is reported which is essential for their growth, the associated epiphytic bacteria may be partly responsible for the production of these substances³⁶. The present study HV10 strain showed wide spectrum antibacterial activity where as other strains showed low to moderate level activity. Similarly Kanagasabhapathy *et al*¹³ reported the antibacterial activity in the supernatants of epiphytic bacteria from red algae such as *Pachymeniopsis lauceolata*, *Plocamium telfairiae*, *Gelidium amansii*, *Chondrus oncellatus*, *Grateloupia filicina*, *Ceramium kondoi*, *Lomentaria catenata*, *Schizymenia dubyi* and *Porphyra yezoensis*. Thus, the seaweed associated epiphytic bacteria was found to have a high percentage of antimicrobial metabolites³⁷. In some isolates of epiphytic bacterial strains, the activity was low against the pathogens. It is possibly due to the fact that these pathogenic bacteria are not natural competitors for the epibiotic bacteria and therefore lack in secondary metabolite that would induce the antimicrobial activity¹³.

The present study, potent active strain (HV10) was identified as *Alcaligenes* sp. based on the colony morphology and biochemical characteristics. This result was supported by Bernan *et al*³⁸ that a most active marine bacteria belongs to *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Vibrio* sp, *Flavobacterium* sp, *Alcaligenes* sp, *Xanthomonas* sp and *Achromobacter* sp.

The subsequent objective of the study was to extract the culture supernatants of predominantly active strain *Alcaligenes* sp (HV10) in three solvents such as hexane, n-butanol and water and assess the antibacterial activity. The n-butanol and water extracts showed wide spectrum activity against bacterial pathogens. The n-butanol extract showed high level inhibition than water extracts. The maximum zone was observed against *Shigella flexneri* followed by *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Salmonella typhi*, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus epidermis*. The present observation was in contrast to Emmanuel *et al*³² reported that the n-butanol extracts of epiphytic bacteria from *Gracilaria edulis* had shown nil activity against the pathogens. This study indicated that the seaweed *Hypnea valentiae* and associated epiphytic bacterial strains offer a potential scope for antibacterial compounds of biomedical importance and further studies are needed to characterize the active compound.

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