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RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Potential of Crude Extracts Derived from Red Algae of Gulf of Mannar

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Abstract

Often referred as the "jewel of the basin" the ocean contains a vast array of pivotal bioactive compounds from both living and non-living sources. The macroalgae have been regarded as a pool diversity of marine environment. They provide number of natural bioactive compounds to alleviate human ailments and thousands of them are isolated from distinct species and various places. Recently pharmacological industries paid attention to discovering various low side effect bioactive compounds from marine sources, especially macroalgae. The present study collected few red algae like *Gelidiella acerosa* (Forsskal), *Acanthophora spicifera* (Vahl) and *Hypnea musciformis* (Wulfen) from the low tide region in Pudumadam coast, Gulf of Mannar; *Centroceras clavulatum* (C. Agardh) and *Gracillaria corticata* (J. Agardh) from Pamban coast, Gulf of Mannar (GoM) and *Galaxaura obtongata* from Villunditirtham coast, Palk Bay and *Laurencia papillosa* (C. Agardh) (Greville) from the Olaikuda coastal region, Rameswaram, Palk Bay, Tamil Nadu, India and subjected for bioactive principles screening using various solvents as these compounds would be of a source for new drug candidates. Further, these seven methanol crude extracts examined for their anti-microbial potential using human pathogens. Our findings revealed stronger anti-microbial potential of methanol extracts of *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis*. The results of the present study confirmed the potential use of marine algae as a reliable source of antibacterial agents. Based on these results, further purification, and identification of individual compounds required as they are specific for anti-microbial activity may pave way for the development of novel therapeutics to treat illness caused by microbes. Besides, further investigations are crucial to elucidate the structure and specific molecular mechanisms of these bioactive components which are responsible for various additional pharmacological activities.

Keywords: Marine Compounds, Antimicrobial, *Gelidiella Acerosa*, *Acanthophora spicifera*, *Hypnea musciformis*

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INTRODUCTION

The marine environment is the jewel of treasury, a valuable and precious resource for diversity of natural products produced by aquatic organisms.[1] Since marine derived bioactive compounds have unique chemical structures, pharmacological industries paid more attention to them due to their critical role in drug discovery and development.[2] In fact, some of them are already under investigation to develop the new pharmaceutical lead compounds/agents[2,3] against malaria, microbes, cancer, inflammation, microtubule, proliferation, hypertension and cytotoxic.[4-9] In recent years, the list grows especially on discovering the low side effect drug leads or compounds.[4] In line with this, hundreds of drug leads were identified and isolated from various marine sources, which include microorganisms, terrestrial plants, sponges, corals, macroalgae, molluscs, echinoderm, etc.[10] The compounds isolated from various marine organisms are of variable chemicals in nature including peptides and phenols.[11]

In general, marine plants (marine macroalgae) are multicellular thallophytes and heterogeneous assemblage of photosynthetic eukaryotic organism.[12] They are classically divided into three main divisions such as green (Chlorophytes), red (Rhodophytes) and brown algae (Phaeophytes).[13] Macroalgae have evolved with an array of complex mechanisms for their survival and resist infections.[14] These qualities make great potential for exploration of bioactive compounds against human illnesses. According to previous reports 3.5 percent extracts from marine origin exhibit toxicity against cancer cells.[15] The isolation and separation of bioactive constituents from marine sources, especially algae, has received tremendous attention. This is due to their crucial role against various diseases like goiter, dysentery and diarrhea.[16] Investigators around the world and pharmaceutical industry are trying to explore the novel mechanisms of many unexplored bioactive principles, especially peptides from marine macroalgae as drug leads or drug candidates.[17] The following macroalgae such as *Macrocysties, Laminaraia, Fucus, Sargassum, Gracillaria, Gelidiella, Hypnea, Turbinaria, Homorphysa* and *Hydrocultres* are of potential interest.[18]

Besides, macroalgae are valuable sources of nutrient rich food ingredients and serving as a unique platform for new biologically functional compounds[14] and minerals.[19-23] Understanding the bio-regulatory function of various endogenous peptides in the organisms and their cellular mechanisms against specific cellular targets would be of promising strategy in drug lead or drug candidate development.[24]

Traditional uses of Macroalgae

For longer-term, macroalgae play crucial roles in the marine ecosystems not only in providing pharmaceutics, agricultural products, biofuels, nutrients, and skin protective care cosmetics,[2,25,26] but also practiced in the Chinese, Korean and Japanese traditional medicine system.[27-31]

Habitat and diversity of macroalgae

There are 30,000 known species of macroalgae reported around the world under three major groups. Approximately 6500 species belonging to Rhodophyta, 1800 species of Phaeophyceae, and 1500 species of Chlorophyta.[32,33] Of these, 145 species are directly used for nutrient purposes, and 24 species are used in complementary and alternative medicine. Besides, around 25 species utilized for cultivation, the preparation of animal feed and fertilizers, and 12 species planted and named as "marine farming".[34,35] India considered as one of the mega-biodiversity habitats for macroalgae and highly regarded as an endangered ecoregions in the world.[36] Moreover, in the Indian coastal waters evidence with more diverged genera (i.e., 217) species (i.e., 844). Amongst the most abundant reported ones Rhodophyta with 434 species, next one is Chlorophyta with 216 species, and Phaeophyta with 191 species and finally Xanthophyte with 3 species.[37] Whereas in the Tamil Nadu coastal regions (South India) inhabited more than 302 species of macroalgae. Nearly 147 species of marine macroalgae (i.e., 42 Chlorophyta, 31 Phaeophyceae, 69 Rhodophyta and 5 blue-green algae) are reported exclusively in the Gulf of Mannar.[38] Besides, Gujarat coastal area have more than 202 species and the presence of macroalgae in other coastal areas of India such as Maharashtra,

Table 1. List of biopotential of macroalgae in Indian coastal regions

No.	Name of the Species	Activity
1	*Asparagopsis taxiformis*	Bio potential
2	*Gracillaria gracilis, G. edulis, Chondrococcus hornemanni, Caulerpa racemosa*	Anti-oxidant
3	*Ulva lactuca, Gracilaria corticata*	Anti-bacterial
4	*Anthophycus longifolius*	Anti-bacteria
5	*Kappaphycus alwarezi*	Anti-oxidant
6	*Laurencia obtus*	Phytochemical
7	*Ulva* spp., *Caulerpa* spp., *Spongomorpha* spp.	Anti-microbial
8	*Gracilaria* sp.	Anti-viral
9	*Sargassum wightii, Kappaphycus alwarezi*	Anti-microbial
10	*Green algae*	Anti-bacterial
11	*Sargassum wightii, Turbinara ornate*	Anti-bacterial
12	*Gracilaria corticata*	Anti-bacterial
13	*Gracilaria corticata*	Anti-microbial
14	*Falkenbergia hillebrandii*	Anti-bacterial
15	*Gracillaria* sp., *Padina* sp., *Sargassum* sp.	Anti-bacterial
16	*S. wightii*	Immunostimulation
17	*Kappaphycus alvarezii*	Anti-oxidant
18	*S. wightii*	Anti-oxidant
19	*Eucheuma denticulatum*	Anti-bacterial
20	*Gelidiella acerosa, Gracillaria verrucosa, Hypnea musciformis*	Anti-bacterial
21	*Ulva fasciata, H. musciformis*	Bio potential
22	*Laurencia* sp.	Anti-bacterial
23	*Spatoglossum variabile*	Bio potential
24	*Turbinaria* spp.	Anti-oxidant

Andhra Pradesh, Goa and Lakshadweep Islands have 159, 79, 75 and 89 species respectively.[39]

Sources of bioactive peptides and other compounds

It is known that biologically active compounds are isolated not only from marine fauna but also from marine flora like macroalgae as potential therapeutics.[17] Macroalgae contributes diverse types of peptides exhibiting various bioactive properties of Garg et al.[40] bacteria and tumor. In fact, proteins like lectins and phycobiliproteins (PBP) of macroalgae substantiated for clinical diagnosis and other applications.[41-43] Bioactive peptides like Ceratospongamide isolated from Rhodophyta, *Ceratodictyon spongiosum* and *Sigmadocia symbiotica* have shown anti-inflammatory properties[44] and Kainoid compounds from Chondria armata perform a key role in neurophysiological syndromes like Alzheimer, Parkinson, and epilepsy recently used as standard therapeutic agent.[45,46] In addition, some red algae have shown to reduce blood pressure and inhibit angiotensin-converting enzyme (ACE) activity.[47-50] Similarly, some species of blue-green algae extracts are employed in complementary and alternative medicine for treating cardiovascular diseases.[51,52] Table 1 describes a list of bio-potential properties of marine macroalgae found in Indian coastal waters.[52] Many bioactive peptides from marine origin are under clinical trials.[6,53] It is well established that microbial infection produces a huge rate of mortality in humans and aquatic organisms.[54] In recent years, the usage of antibiotics increased significantly owing to various types of microbial infections and the pathogenic microbes becoming resistant to drugs is common due to indiscriminate use of antibiotics.[55] It becomes a greater problem of giving proper treatment against resistant pathogenic organisms. In line with this, many researchers and pharmaceutical companies are showing keen interest in isolation of various peptides from red algae because of their easy cultivation with cheaper cost, low side

effects and for their potency[56] specifically on antimicrobial properties related to marine algae against several pathogens.[57] Besides, marine algae represent an inexhaustible reservoir of raw materials used in pharmaceutical, medicine, food and cosmetics industries.[58] Although various antimicrobial compounds derived from marine algae extracts consist of a diverse group of chemical constituents[59] and they have exhibited antibacterial activity against microbes[60,61] yet a limited data is available on red algae derived compounds/peptides from the Indian coastal waters specifically the Gulf of Mannar and Palk Bay Coasts, which encouraged the current investigation to collect, prepare the extract and identify bioactive peptides from the selected red algae of Gulf of Mannar regions (Figure 1) and examined antimicrobial properties of their crude extracts. Phytochemical screening and identification of bioactive constituents may help the drug discovery and development processes which include antimicrobial agents.

MATERIALS AND METHODS

Study area

Gulf of Mannar (GoM) is the study area for this investigation; it is in the southern east coastal region of India; it is transitional zone between Arabian Sea and Indian Ocean. Palk Strait connects Gulf of Mannar with Bay of Bengal. (Figure 2). It is the first Marine Biosphere Reserve in Southeast Asia with the greatest marine biodiversity in the world and comprises of various marine components like mangroves, algal communities, seagrass beds, coral reefs, and salt marshes.[62]

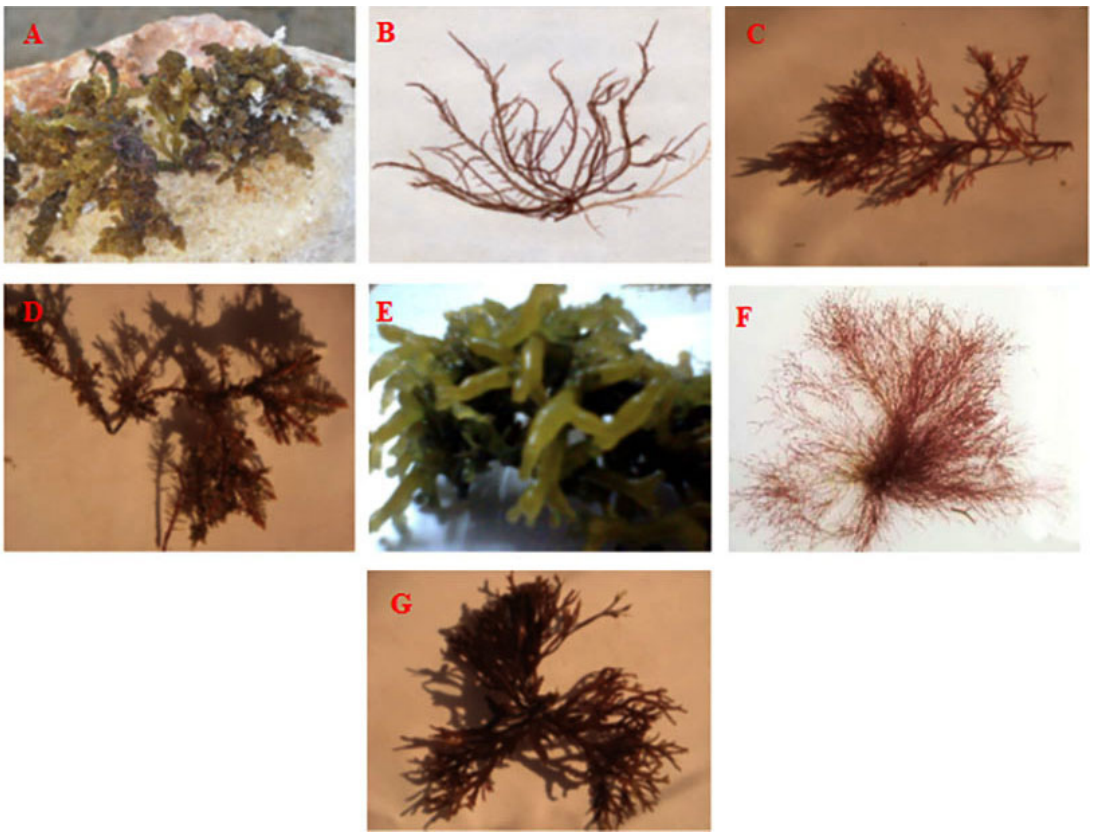

Figure 1. Macroalgae/Seaweed used for bioactive principal extraction, screening, and anti-microbial evaluation. (A) *Laurencia papillosa,* (B) *Hypnea musciformis,* (C) *Acanthophora spicifera,* (D) *Gelidiella acerosa,* (E) *Galaxaura oblongata,* (F) *Centroceras clavulatum,* (G) *Gracilaria corticata*

Gulf of Mannar (Biologists paradise) has more than 3600 plant and animal species. The Gulf of Mannar's diverse ecosystems support a widespread diversity of marine organisms including macroalgae: more than 147 species (Chlorophyta 42 species, Phaeophyceae 31 species, Rhodophyta 69 species and blue-green algae 5 species). This coastal region also contains other alga types like red and brown.[63] Palk Bay and Gulf of Mannar separated by Mandapam peninsula and Rameswaram Island. There is only fringing reef found in the Palk Bay. So far, 117 species of corals reported in both regions, in which Palk Bay region alone contains more than 63 species of scleractinian corals.[64,65]

Sample collection

In the present investigation, macroalgae like *Gelidiella acerosa*,[66] *Acanthophora spicifera*[67] and *Hypnea musciformis*,[68,69] were collected from low tide region in Pudumadam coast *Centroceras clavulatum* (C. Agardh) and *Gracillaria corticata* (J. Agardh) from Pamban coastal region of Gulf of Mannar), and *Galaxaura oblongata* from Villunditirtham coast, Palk Bay whereas *Laurencia papillosa* (C. Agardh) (Greville) was from Olaikuda coastal region, Rameswaram Palk Bay, southeast coast of India (Table 2). The collected macroalgae rinsed to remove epiphytes and other impurities and kept sterile in the laboratory located at Pudumadam, Ramanathapuram District, Tamil Nadu for taxonomy identification and for further experiments.[70]

Preparation of crude extract

The cleaned macroalgae allowed for shadow dry and powdered using mixer grinder. Ten (10) gm powder from each sample suspended in methanol (Merck AR Grade) and stored in a horizontal shaker (Remi) at room temperature and centrifuged to collect the supernatant and pooled. Methanol solvent extracts dried under nitrogen flow using a rotary vacuum evaporator (Puchi RII, Switzerland). To avoid degradation, concentrated pooled extract stored in sterile, airtight containers at -20 °C until further use.[71,72]

Bioactive compounds screening

Seven potential red algae extracts selected and subjected for bioactive principles screening. Mayer's and Dragendorff's tests employed alkaloids, terpenoids, tannins, and glycosides identification. In addition, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay used to assess the phenolic content.[73]

Figure 2. Map of study area of Gulf of Mannar and Palk Bay Coast, Southeast Coast of India, International Maritime Boundary Line (IMBL)
IUCN-MMPATF.Gulf of Mannar and Palk Bay IMMA Factsheet. IUCN Joint SSC/WCPA Marine Mammal Protected Areas Task Force, 2022

Table 2. Classification of algae species

Classification	*Gelidiella Acerosa*	*Acanthophora Spicifera*	*Hypnea Musciformis*
Phylum: Rhodophyta			
Sub Phylum: Eurhodophytina			
Class: Florideophyceae			
Sub Class: Rhodymeniophycidae			
Order:	Gelidiales	Ceramiales	Gigartinales
Family:	Gelidellaceae	Rhodomelaceae	Cystocloniaceae
Genus:	Gelidiella	Acanthophora	Hypnea
Species:	acerosa	spicifera	musciformis

Preparation of bacterial culture and anti-microbial evaluation

The bacterial strain individually inoculated in the test tube containing nutrient broth incubated at 28 ± 2 °C for 24 hrs. After that, the autoclaved Mueller-Hinton agar medium transferred aseptically into sterile Petri dish (approximately 20 mL per dish). Finally, the medium allowed to solidify. On this, the bacterial broth culture inoculated by using 'L' rod.

The crude extract which showed positive results on bioactive principles only evolved for the

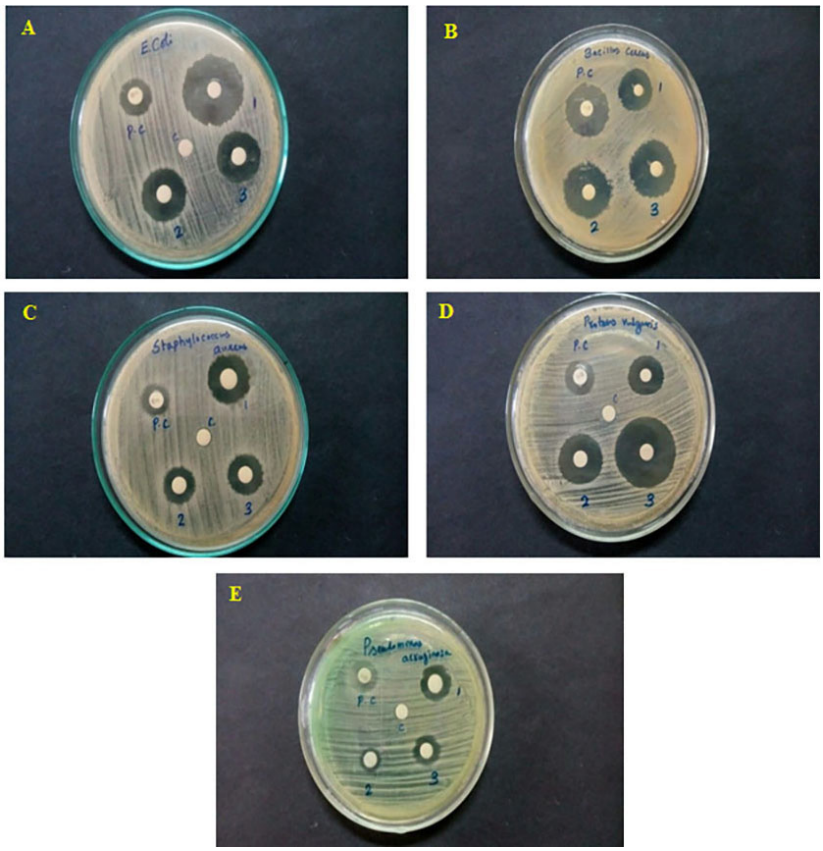

Figure 3. Zone of inhibition against bacterial pathogens exposed to crude extracts of *Gelidiella acerosa* (1), *Hypnea musciformis* (2) and *Acanthophora spicifera* (3); PC - Postive Control. (A) *Escherichia coli*, (B) *Bacillus cereus,* (C) *Staphylococcus aureus,* (D) *Proteus vulgaris*, (E) *Pseudomonas aeruginosa*

disc diffusion method as it is a qualitative and semi-quantitative test for determining antimicrobial susceptibility experiment.[74] The antibacterial (disc diffusion method) test performed on both Gram-positive and -negative bacteria using red algae crude extracts at different concentration between 6.5-25 µg/mL This method is used to follow pathogenic microorganisms: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus,* and *Proteus vulgaris*.[74,75] The sterile disks individually soaked with crude extracts of each algal sample. Agar plates inoculated uniformly from the broth culture of tested microorganisms. The methanol crude extract of each algal species applied to a separate set of sterile disks using uniform inoculation of agar plates containing tested microorganisms. Streptomycin was employed as a positive control (PC) and methanol devoid of algae extract used as negative control (NC). The experiment runs duplicate, with clear radiance (zone formation).[76] This study conducted on marine red algae for bioactive principles screening and for testing antimicrobial potential using crude extracts hence this study requires no ethical approval.

Statistical analysis

To validate data robustness and reproducibility, experiments conducted separately in triplicate. All data expressed as mean values ± SD, the mean values analyzed by three ways analysis of variance using the Statistical Analysis System (SAS) (1997) computer program.[77]

RESULTS

Red algae extract yield and phytochemical composition

This study used various solvents like polar (Ethanol, Methanol and Acetone) and non-polar (chloroform and hexane) for extract preparation. Amongst tested solvents methanol generated the maximum extract quantity which is sufficient for successive biological and phytochemical analyses. Table 3 summarizes the phytochemical screening results of the algae extracts, which display different compound profiles.

Anti-microbial activity evaluation

To determine whether the seaweed extracts had any inhibitory potential, screening

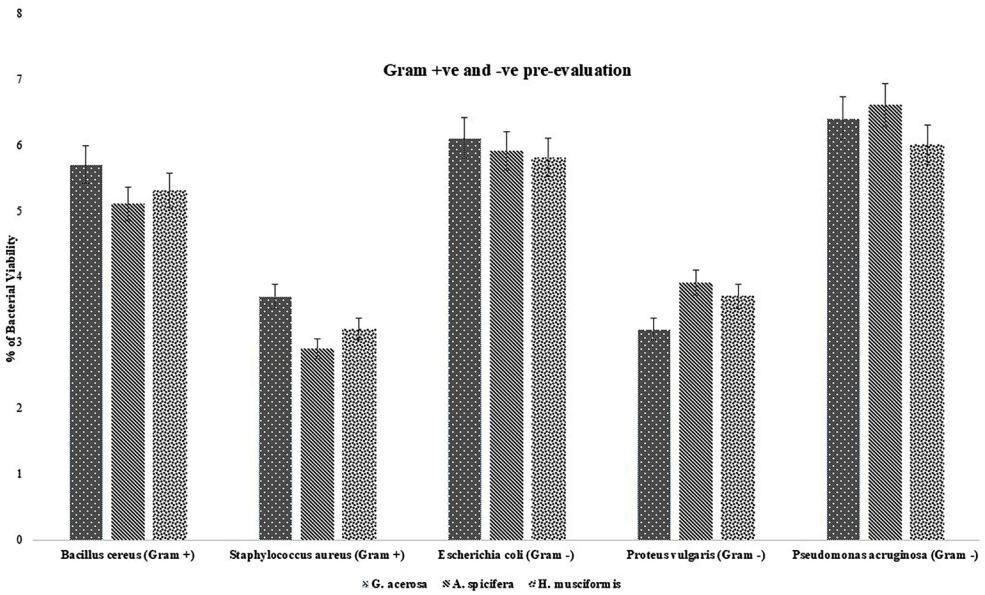

Figure 4. Pre-evaluation of the anti-microbial activity of methanol extract (25 µg/mL of each extract) of *G. acerosa, A. spicifera* and *H. musciformis* against pathogenic extracts against Gram-positive and Gram-negative bacteria

Table 3. Phytochemical analysis of *G. acerosa, A. spicifera* and *H. musciformis*

Phytochemicals	*G. acerosa*	*A. spicifera*	*H. musciformis*
Tannins	++	+++	-
Alkaloids	-	-	-
Flavonoids	+++	++	++
Saponins	-	-	-
Phytosterol	+++	+++	-
Glycoside	+++	+++	+
Oils & Fats	++	++	-
Protein	+++	+++	++
Carbohydrates	+++	+++	+++
Resins	++	++	-
Coumarins	++	++	+
Terpenoids	+++	+++	-

+ Weak (<3 µg/ml), ++ Moderate (5-7 µg/ml), +++ Strong (>8 µg/ml), - Absent

was carried out using *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Proteus vulgaris* by evaluating their growth potential in wells containing seaweed extract and bacterial suspension (extract concentrations ranging from 6.5, 12.5 and 25 µg/mL, respectively). Controls prepared containing broth and bacterial suspension (positive control), or broth only (negative control).

Anti-microbial activity of crude extract of *Gracilaria corticata, Gelidiella acerosa, Galaxaura oblongata, Centroceras clavulatum, Laurencia papillosa, Hypnea musciformis* and *Acanthophora spicifera* samples evaluated against five human pathogens by disk diffusion method. The Kirby-Bauer disk diffusion susceptibility test,[78] generally employed to ascertain how sensitive or resistant pathogenic microorganisms are to various antimicrobial lead compounds.[79] Out of seven red algae samples tested the following three species such as *Gelidiella acerosa, Acanthophora spicifera* and *Hypnea musciformis* only showed efficient activity (zone formation) against anti-microbial pathogens. However, the other methanol extracts of *Gracilaria corticata, Galaxaura oblongata, Centroceras clavulatum* and *Laurencia papillosa* showed less or no activity (negative result) (i.e., zone of inhibition not noticed) (Table 4 and Figure 3). Although, the present investigation used various extract concentrations ranging between 6.5, 12.5 of and 25 µmg/mL for the disc diffusion, the higher concentration (25 µmg/mL) has shown the good inhibitory zone, whereas the other lower concentrations exhibited less visible/no demarkable zone, hence this study adopted 25 µmg/mL as the only test concentration against bacterial strains.

The zone of inhibition against various bacterial pathogens by marine algae extracts from *G. acerosa, A. spicifera* and *H. musciformis* described in Table 5. The algae *Gelidiella acerosa* exhibited an inhibitory zone of 16 mm, 11 mm, 16 mm, 7 mm, and 11 mm against *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, *Pseudomonas acruginosa,* and *Proteus vulgaris,* respectively but only 7 mm against *P. aeruginosa*. For *Acanthophora spicifera*, the zones of inhibition measured were 10 mm against *Escherichia coli,* 8 mm for *Staphylococcus aureus,* 22 mm for *Bacillus cereus,* and 13 mm for *Proteus vulgaris,* whereas the *Hypnea musciformis* exhibited 13 mm for *Escherichia coli,* 10 mm for *Staphylococcus aureus*, 26 mm for *Bacillus cereus,* and 20 mm for *Proteus vulgaris.* The zone of inhibition against *Pseudomonas aeruginosa* was very less in *Acanthophora spicifera* and *H. musciformis,* i.e. 2 mm and 5 mm, respectively. The above stated findings clearly showed that *Gelidiella acerosa* had the strongest activity against *Bacillus cereus* and *Escherichia coli* (i.e. 16 mm ZOI); whereas *Acanthophora spicifera* exhibited its activity against *Bacillus cereus* with the highest ZOI (22 mm) and *Hypnea musciformis* crude extract revealed the maximum ZOI (26 mm) against *Bacillus cereus.* To support further, the pre-evaluation of the anti-microbial activity of methanol extract (25 mg/

Table 4. Anti-microbial activity of seven species of red algae against bacterial pathogens

Bacterial strains	*G. acerosa*	*G. corticata*	*G. oblongata*	*C. clavulatum*	*L. papillosa*	*A. spicifera*	*H. musciformis*
E. coli	++	–	–	–	–	++	++
B. cereus	++	–	–	–	–	++	++
S. aureus	++	–	–	–	–	++	++
P. vulgaris	++	–	–	–	–	++	++
P. aeruginosa	++	–	–	–	–	++	++

+ Activity; – No activity

Table 5. Zone of inhibition by marine algae extracts from *G. acerosa, A. spicifera* and *H. musciformis*

No.	Bacterial strains	Algae	Solvent	NC	PC	Extract Conc (µg/mL)	Zone of inhibition (mm)
1	*E. coli*	*G. acerosa*, *A. spicifera* and *H. musciformis*	M	Nil	0.4	25	161013
2	*B. cereus*	*G. acerosa*, *A. spicifera* and *H. musciformis*	M	Nil	1.0	25	162226
3	*S. aureus*	*G. acerosa*, *A. spicifera* and *H. musciformis*	M	Nil	0.3	25	11810
4	*P. vulgaris*	*G. acerosa*, *A. spicifera* and *H. musciformis*	M	Nil	0.3	25	111320
5	*P. acruginosa*	*G. acerosa*, *A. spicifera* and *H. musciformis*	M	Nil	0.3	25	725

NC: Negative Control (broth), PC: Positive Control (broth + bacteria suspension), M: Methanol

Table 6. Pre-evaluation of the anti-microbial activity of methanol extract (25 µg/mL of each extract) of *G. acerosa, A. spicifera* and *H. musciformis* against pathogenic extracts against (Gram-positive) and (Gram-negative) bacteria

No.	Bacterial strains	*G. acerosa*	*A. spicifera*	*H. musciformis*
		% of bacterial viability ± SD		
1	*Escherichia coli* (Gram -)	6.1 ± 2.4	5.9 ± 2.1	5.8 ± 1.7
2	*Bacillus cereus* (Gram +)	5.7 ± 2.5	5.1 ± 1.3	5.3 ± 1.9
3	*Staphylococcus aureus* (Gram +)	3.7 ± 1.3	2.9 ± 2.1	3.2 ± 1.8
4	*Proteus vulgaris* (Gram -)	3.2 ± 2.7	3.9 ± 2.1	3.7 ± 2.1
5	*Pseudomonas acruginosa* (Gram -)	6.4 ± 3.2	6.6 ± 2.7	6.0 ± 1.9

SD = Standard deviation

mL) of *Gelidiella acerosa, Acanthophora spicifera* and *Hypnea musciformis* done against pathogenic bacteria shown in Table 6 and Figure 4.

DISCUSSION

It is well known that bacterial infection causes high mortality among humans, animals, and aquatic organisms because many microorganisms like *Bacillus subtilis* which are accountable for foodborne gastroenteritis, E*scherichia coli* for mastitis and for many other harmful effects.[80] *Staphylococcus aureus* causes abortion whereas *Pseudomonas aeruginosa* causes upper respiratory complications and fatal illness, while *Salmonella* sp. produces diarrhea and typhoid fever.[81,82] The prevention/treatment of diseases dealt with many known drugs, chemicals or antibiotics however these pathogenic microorganisms become more resistant to these drugs indiscriminately.[83] Moreover, antibiotic medications are becoming increasingly expensive and produce hypersensitivity for some and cause reduction in beneficial microorganisms in the gut and produce unfavorable effect on the host.[84]

Marine entities are rich sources of biologically active and structurally unique metabolites.[85] Marine organisms capable of producing both primary and secondary metabolites, which are of interest to many pharmaceutical companies as drug leads/ candidates.[86] Numerous researchers are interested in developing new alternative bioactive principles with low cost but high efficiency against resistant pathogens.[2,87-89] In fact, some of unique new therapeutic leads, natural products of marine origin with a variety of biological activities have been screened and isolated.[90] In line with this, it has been demonstrated that the active components and different algae extracts have revealed antimicrobial activity against a variety of pathogens including bacteria.[10]

The disc diffusion method is a qualitative and semi-quantitative test adopted by many studies for determining antimicrobial susceptibility, predominantly employed in natural products research.[91,92] The present study adopted this diffusion method to screen seven red algal species, which include *Gelidiella acerosa, Acanthophora spicifera, Hypnea musciformis, Gracilaria corticata, Galaxaura oblongata, Centroceras clavulatum* and *Laurencia papillosa* crude extracts to test their qualitative anti-microbial potential. The methanol extract of *Gracilaria corticata, Galaxaura oblongata, Centroceras clavulatum* and *Laurencia papillosa* samples were unable to exhibit anti-microbial impact on pathogenic microorganisms like as *Pseudomonas acruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus,* and *Proteus vulgaris* while the remaining three species exhibited good antibacterial properties. The above results suggest that microbial pathogens are exhibiting mechanisms against resistance through intracellular drug accumulation, enzymatic inactivation, and modification of target sites and decrease and insufficient drug concentration.[93,94]

In line with previous investigations, the current study confirmed that the methanol extraction yields higher and showed effective anti-microbial activity than other organic solvents used.[5] The use of methanol solvent extraction always provides higher efficiency and yielded anti-microbial compounds.[5,95-98] To support further, the shade dried macroalgae extracts had privileged action on microbial pathogens than that of fresh macroalgae extracts[99] as the extract obtained from fresh macroalgae samples exhibited lower anti-microbial activity.[100]

The present results obtained with methanol extracts of *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis* collected from Gulf of Mannar coastal regions are comparable to those with different species collected from the Canary Islands against various bacteria.[101] Further evidence suggest that the methanol-dichloromethane extracts of *Hypnea musciformis* from the Indian coast are more effective against pathogenic bacterial strains.[102] In line with this, numerous reports are aligned and supported that the methanol extract of *Gelidiella acerosa*, *Acanthophora spicifera* as well as *Hypnea musciformis* exhibits strong antimicrobial potential against a variety of bacterial strains.[102,103] Hence, the present study outcomes clearly indicate and confirm that the methanol extract of *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis* have shown significant antimicrobial activity against bacterial strains.

CONCLUSION

The present investigation outcome with anti-microbial activities observed in crude methanol extracts of *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis* gathered from Pudumadam, Gulf of Mannar, southeast coast of India provides an excellent indication that algae maintain an effective antimicrobial properties against *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Proteus vulgaris* bacterial strains and this antibacterial effect may be due to the presence of various prospective natural compounds. The present study concludes that the red algae, *Gelidiella acerosa, Acanthophora spicifera* and *Hypnea musciformis* are potential sources of bioactive compounds that evoked strong antibacterial activities, which utilized for the development of various therapeutic agents including natural antibiotics against multidrug-resistant bacteria. However, further investigations required structural elucidation as well as specific molecular mechanisms of action which are responsible for various additional pharmacological activities. The characterization of various bioactive

components obtained from these macroalgae may pave way for the development of more novel therapeutics by the pharmaceutics industry as they may contain unexplored health benefits, in addition to their previously well demonstrated nutritional blended in the Eastern as well as Western traditional cuisines. The adaptation of algal nutrition is now becoming a global trend in the human diet.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

CMR conceptualized the study and provided guidance. KDK performed experiments and analyzed. AAYA performed statistical analysis. EMMH, RYY and MAS wrote the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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