

# Phytochemical identification of compounds using HR-LCMS analysis and antioxidant as well as antibacterial activity of aqueous extracts of Seaweeds.

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## Abstract

The diverse array of phytoconstituents in marine macro algae highlights their potential as functional foods and sources of natural bioactive compounds for health applications. The aim of this study was to find different bioactive compounds and evaluate their antioxidant qualities and antimicrobial effects in *Gracilaria corticata*, *J. Ag.*, *Padina tetrastromatica* Hauck and *Caulerpa peltata*. Lamour. The process included gathering the seaweed, washing it with flowing water, drying it in the shade and then extracting it with water. The phytochemical analyses conducted on the three varieties of seaweeds showed the existence of phenols, flavonoids, alkaloids, saponins, tannins and glycosides, through both qualitative and quantitative methods. Nonetheless, terpenoids were not present in *Gracilaria*. HRLC-MS was utilized to examine the phytochemical compounds found in the seaweed. The disc diffusion technique was used to evaluate the antimicrobial efficacy of seaweed extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*. HR-LCMS analysis revealed diverse compounds in both the positive and negative chromatogram. The water extract from *Gracilaria corticata* showed higher antioxidant activity than the other extracts evaluated. The largest areas of inhibition were seen in the presence of the gram-positive bacteria *Staphylococcus aureus* ( $15.33 \pm 1.15$ ) and gram-negative bacteria *Klebsiella pneumoniae* ( $20.67 \pm 1.15$ ).

**Key words:** Seaweeds; phytoconstituents; Antioxidant; HRLC-MS; Anti-bacterial

## 1. Introduction

Marine macroalgae have emerged as significant natural resources due to their ability to synthesize a wide array of chemically diverse compounds with functional relevance (Dhargalkar & Pereira, 2005; Holdt & Kraan, 2011). These organisms produce numerous primary and secondary metabolites including aromatic compounds, nitrogen-containing molecules and complex organic constituents which contribute to their ecological resilience and biological activity (Plaza et al., 2008). Such compounds not only support algal

survival in dynamic marine environments but also provide valuable properties that can be exploited for biomedical and industrial applications (Holdt & Kraan, 2011).

One of the most important functional attributes of marine algae is their capacity to counteract oxidative processes (Cornish & Garbary, 2010). Compounds capable of mitigating oxidative reactions help protect algal cells from damage caused by environmental stressors such as high light intensity and oxygen-rich conditions (Heo et al., 2005). These antioxidative mechanisms have gained attention because similar processes are involved in preventing cellular damage in biological systems (Wong et al., 2000). As a result, seaweed-derived antioxidant molecules are increasingly investigated for their potential use in health-related products and natural preservation systems (Plaza et al., 2008; Cornish & Garbary, 2010).

Beyond their antioxidant role marine macroalgae are also known to exhibit inhibitory effects against a variety of microorganisms (Bansemir et al., 2006). Numerous studies have demonstrated that extracts obtained from different algal groups possess activity against bacterial and fungal pathogens (Manilal et al., 2010). This biological activity is attributed to the presence of structurally unique metabolites that interfere with microbial growth and metabolism (Chew et al., 2011). The expanding need for alternative antimicrobial agents has further increased interest in marine algae as sustainable and environmentally compatible sources of bioactive compounds (Bansemir et al., 2006; Manilal et al., 2010).

Based on these considerations, the present study focuses on the qualitative assessment of bioactive constituents present in aqueous extracts of three marine macroalgae—*Gracilaria corticata* J. Ag., *Padina tetrastromatica* Hauck, and *Caulerpa peltata* Lamour—and evaluates their antioxidant and antimicrobial properties.

## **2. Materials and methods**

### **Collection and authentication of algal samples**

Marine macroalgae were collected from the Thikkodi coastal region of Kozhikode district, Kerala (11°29' N latitude and 75°37' E longitude) during November 2019. After collection, the samples were thoroughly washed to remove sand, epiphytes and extraneous matter followed by shade drying at ambient temperature. Taxonomic identification of the algal species was carried out using standard taxonomic keys and reference literature (Desikachary et al., 1990; Desikachary et al., 1998; Krishnamurthy, 2000; Krishnamurthy and Baluswami, 2010).

### **Preparation of aqueous extracts**

The dried algal material was pulverized to obtain a fine powder. Extraction was performed using distilled water as the solvent. The resulting extracts were filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrates were preserved under refrigerated conditions until further experimental analysis.

### **Qualitative phytochemical study**

Preliminary phytochemical screening was carried out on the aqueous algal extracts to detect the presence of major classes of secondary metabolites. Standard qualitative assays were employed to identify bioactive

compounds such as phenols, flavonoids, alkaloids, saponins, tannins, terpenoids and glycosides following established protocols (Harborne, 1984; Kokate, 1994).

### **Quantitative phytochemical study**

#### **Ascertainment of total phenolic content**

Total phenolic content of the aqueous extracts was estimated using the Folin–Ciocalteu reagent method. Absorbance values were measured at 760 nm using a spectrophotometer, following standard procedures (Alhakmani et al., 2013; Ainsworth and Gillespie, 2007).

#### **Ascertainment of total flavonoid content**

Flavonoid concentration in the extracts was quantified using the aluminium chloride colorimetric method as described by Bag and Devi (2015).

#### **Estimation of saponin content**

Saponin levels were determined using the vanillin–sulphuric acid assay according to the method proposed by Simee (2011).

#### **Estimation of alkaloid content**

Total alkaloid content was measured following the procedure described by Evans (1966).

#### **Determination of tannin content**

Tannin concentration was quantified using the Folin–Denis reagent method in accordance with Sastri (1962) and Schandrei (1970).

#### **Estimation of total terpenoids**

The terpenoid content present in the aqueous extracts was analyzed using the method described by Ghorai (1996).

#### **Estimation of glycoside content**

Quantitative estimation of glycosides was performed following the protocol outlined by El-Olemy et al. (1994).

#### **Antibacterial activity assay**

The antibacterial potential of the aqueous algal extracts was assessed using the disc diffusion technique. Gram-positive and Gram-negative bacterial strains were obtained from Sudharma Polyclinic, Thrissur, Kerala. Sterile filter paper discs (6 mm diameter) prepared from Whatman No. 1 paper were used for the assay. Amoxicillin served as the positive control, while methanol was used as the negative control. The procedure was carried out following established methods (Rameshkumar and Sivasudha, 2012).

#### **Antioxidant activity – DPPH radical scavenging assay**

The free radical scavenging activity of the aqueous extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Absorbance was recorded spectrophotometrically, with ascorbic acid

used as the reference standard (Aryal et al., 2019). All experiments were conducted in triplicate. The percentage inhibition of DPPH radicals was calculated using the following equation:

Percentage of inhibition of DPPH radical scavenging activity =

$$\frac{(\text{OD of Control} - \text{OD of Sample})}{\text{OD of Control}} \times 100$$

### HR-LCMS Q-TOF analysis

High-resolution liquid chromatography–mass spectrometry (HR-LCMS Q-TOF) was employed to characterize the chemical constituents present in the aqueous extracts of the three macroalgae. The analysis was carried out at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Mumbai. An Agilent G6550A system with a mass resolution of 0.01% was used for analysis. The Q-TOF mass analyzer enabled accurate molecular mass determination and improved compound identification through molecular formula prediction and library matching of unknown constituents present in the crude extracts.

### Statistical analysis

Data are expressed as mean  $\pm$  SE from three independent experiments.

## 3. Result

Preliminary phytochemical screening of the aqueous extracts of the selected seaweeds was performed to identify the presence of major secondary metabolites with potential biological relevance. The analysis confirmed the presence of phenols, flavonoids, saponins, alkaloids, tannins and glycosides in the algal extracts. These metabolite groups are widely associated with antimicrobial, anti-inflammatory and cytoprotective activities. The qualitative phytochemical profile is presented in Table 1. Terpenoids were not detected in *Gracilaria corticata* whereas steroidal compounds were observed in *Caulerpa peltata*. In contrast, coumarins, quinine, anthraquinones, phlobatannins and cardiac glycosides were absent in all three algal species.

Quantitative estimation of seven phytochemical constituents—phenols, flavonoids, alkaloids, saponins, terpenoids, tannins and glycosides—was carried out and the results are summarized in Table 2. The brown alga *Padina tetrastratica* exhibited the highest total phenolic content ( $100.44 \pm 0.24 \mu\text{g}/\text{mg}$ ). Terpenoids were completely absent in *Gracilaria corticata*. Elevated levels of flavonoids ( $91.26 \pm 0.48 \mu\text{g}/\text{mg}$ ), tannins ( $79.78 \pm 2.10 \mu\text{g}/\text{mg}$ ) and terpenoids ( $163.70 \pm 2.80 \mu\text{g}/\text{mg}$ ) were recorded in the green alga *Caulerpa peltata*. In contrast, *Padina tetrastratica* showed higher concentrations of saponins ( $176.56 \pm 0.96 \mu\text{g}/\text{mg}$ ), glycosides ( $89.82 \pm 0.20 \mu\text{g}/\text{mg}$ ) and alkaloids ( $90.32 \pm 0.35 \mu\text{g}/\text{mg}$ ).

The antioxidant activity of the aqueous algal extracts was assessed using the DPPH free radical scavenging assay. A concentration-dependent scavenging response was observed for all samples. Among the tested extracts, *Gracilaria corticata* exhibited the highest scavenging activity ( $19.44 \mu\text{g}/\text{mg}$ ), followed by *Padina tetrastratica* ( $54.22 \mu\text{g}/\text{mg}$ ) and *Caulerpa peltata* ( $60.27 \mu\text{g}/\text{mg}$ ), as shown in Table 3. However, the scavenging capacity of all algal extracts remained lower than that of the standard antioxidant, ascorbic acid.

The antimicrobial activity of the aqueous extracts was evaluated against selected Gram-positive and Gram-negative bacterial strains and the results are presented in Table 4. An increase in extract concentration resulted in enhanced inhibitory activity against all tested microorganisms. The strongest antibacterial effect was observed for the aqueous extract of *Gracilaria corticata* against *Staphylococcus aureus* ( $15.33 \pm 1.15$  mm) and *Klebsiella pneumoniae* ( $15.67 \pm 1.15$  mm). Comparative analysis revealed that all three algae produced similar zones of inhibition against both Gram-positive and Gram-negative bacteria.

HRLC-MS-Q-TOF analysis of the aqueous extract of *Gracilaria corticata* revealed the presence of fourteen compounds in the positive ionization chromatogram within a retention time range of 1.11–10.811. Among these, Lactapiperol C exhibited the highest mass (282.1799), along with a notable abundance of 3 $\beta$ ,6 $\beta$ -Dihydroxynortropane. In the reverse-phase chromatogram, Cryogene, Physapubenolide and 14,19-Dihydroaspidospermatine were identified at retention times of 10.219, 16.621 and 23.447 respectively. Analysis of *Padina tetrastromatica* identified six compounds in the positive chromatogram with Pirbuterol eluting at the shortest retention time (4.61) and Zanthobisquinolone at the longest (12.632). Zeylenol was detected in the negative ion mode with a retention time of 13 and a mass of 384.1189. HRLC-MS-Q-TOF profiling of *Caulerpa peltata* revealed eleven compounds including the steroid 27-Nor-5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24,25-pentol which showed the longest retention time (18.001) and 8-Hydroxy-2-chlorodibenzofuran which eluted earliest (0.807). Physapubenolide, Dehydrocarpaine II and 14,19-Dihydroaspidospermatine were also detected in the reverse-phase chromatogram due to their higher retention times. The detailed compound profiles are presented in Tables 5–7 and Figures 1–3.

Table 1: Qualitative Phytochemical analysis

Sl.No.	Constituents	Chemical tests	Water Extracts		
			<i>Gracilaria corticata</i>	<i>Padina tetraströmatica</i>	<i>Caulerpa peltata</i>
1.	Alkaloid	Mayer's Test	-	-	-
		Dragendroff's Test	+	+	+
		Hager's Test	-	-	+
		Wagner's Test	+	+	+
		Tannic acid Test	+	+	+
2.	Phenols	Ferric chloride Test	-	-	-
		Lead acetate Test	+	+	+
		Gelatin Test	-	-	-
		Potassium Dichromate Test	+	+	+
		Alkaline Reagent Test	-	-	-
3.	Flavonoid	Alkaline Reagent Test	-	-	+
		Shinoda Test	-	-	-
		Ethyl Acetate-Ammonia Test	+	-	-
		Pew's Test	-	+	-
4	Tannins	Ferric chloride Test	-	+	+
		Lead acetate Test	+	+	+
		Potassium Dichromate Test	+	-	+
5	Saponin	Frothing Test	+	+	+
		Sodium bicarbonate Test	+	+	+
6	Terpenoid	Salkowski's Test	-	+	+
		Hesse's Reaction	-	+	+
7	Glycosides	Keller Killiani Test	-	-	-
		Borntragers Test	-	-	-
		Modified Borntragers Test	+	+	+
		Legal Test	-	-	-
8	Steroids	Salkowski's Test	-	-	+
		Libermann-Sterol Test	-	-	-
9	Coumarin	1 ml Extract + 1ml 10% NaOH	-	-	-
10	Quinine	1 ml Extract + Alcoholic KOH	-	-	-
11	Anthraquinone	1 ml Extract macerate with ether and add aq. Ammonia	-	-	-
12	Cardiac glycosides	2ml extract + 1ml glacial acetic acid + 1 ml Ferric chloride + 1ml conc. H <sub>2</sub> SO <sub>4</sub>	-	-	-

13	Phlobatannin	Extract + distilled water + heated with 2% HCl	-	-	-
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(+) present, (-) absent.

Table 2: Quantitative Phytochemical analysis

Phytochemical constituents	Water extracts		
	<i>Gracilaria corticata</i> (µg/mg)	<i>Padina tetrastromatica</i> (µg/mg)	<i>Caulerpa peltata</i> (µg/mg)
Phenol	73.65±0.31	100.44±0.24	84.14±0.43
Flavonoid	38.08±0.13	38.86±0.47	91.26±0.48
Alkaloid	41.39±0.46	90.32±0.35	21.92±0.57
Saponin	56.56±4.19	176.56±0.96	82.11±0.96
Tannin	68.67±0.84	69.22±1.27	79.78±2.10
Terpenoid	-	96.67±2.23	163.70±2.80
Glycoside	87.92±0.10	89.82±0.20	85.72±0.17

Table 3: Anti-oxidant activity of seaweed

Concentration	Scavenging activity percentage of water extract			
	Ascorbic acid	<i>Gracillaria corticate</i> (%)	<i>Padina tetrastromatica</i> (%)	<i>Caulerpa peltate</i> (%)
100	5.07 ± 0.20	44.59±1.86	35.23±1.46	22.85±2.40
200	18.69 ± 0.49	50.33±1.78	38.41±2.34	28.25±2.47
300	32.21 ± 0.30	56.08±1.73	41.58±3.25	33.65±2.64
400	46.28 ± 0.78	61.82±1.70	44.76±4.19	39.04±2.88
500	59.12 ± 0.39	68.69±2.45	48.99±4.08	43.38±2.11
IC50 VALUES	85.76 (µg/mg)	19.44 (µg/mg)	54.22 (µg/mg)	60.27 (µg/mg)

Table 4: Anti-microbial activity of seaweed

Concentration	<i>Gracillaria corticata</i> (mm)		<i>Padina tetrastromatica</i> (mm)		<i>Caulerpa peltata</i> (mm)	
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Positive control	17.33±1.15	17.67±0.58	19.33±1.15	20.00±2.00	18.33±0.58	18.33±0.58
Negative control	NZ	NZ	NZ	NZ	NZ	NZ

1 mg/ml	10.67±1.15	12.67±1.15	8.33±0.58	8.67±1.15	10.33±1.53	9.67±0.58
2.5 mg/ml	12.67±1.15	14.67±1.15	8.00±1.00	8.33±0.58	12.67±1.15	11.33±0.58
5 mg/ml	15.33±1.15	15.67±1.15	10.33±0.58	10.33±0.58	14.00±1.00	13.33±1.53

Fig 1.1: Positive chromatogram of *Gracilaria corticata*

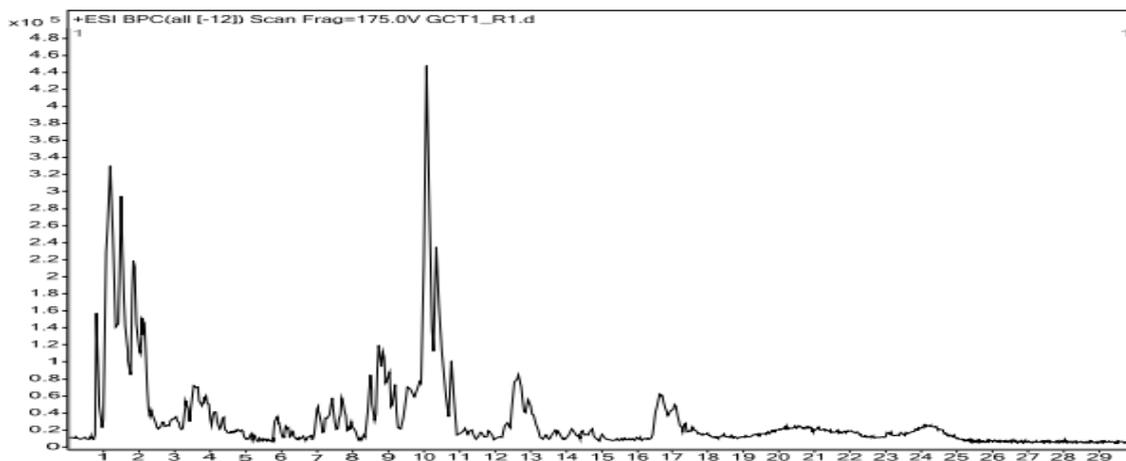


Fig 1.2: Negative chromatogram of *Gracilaria corticata*

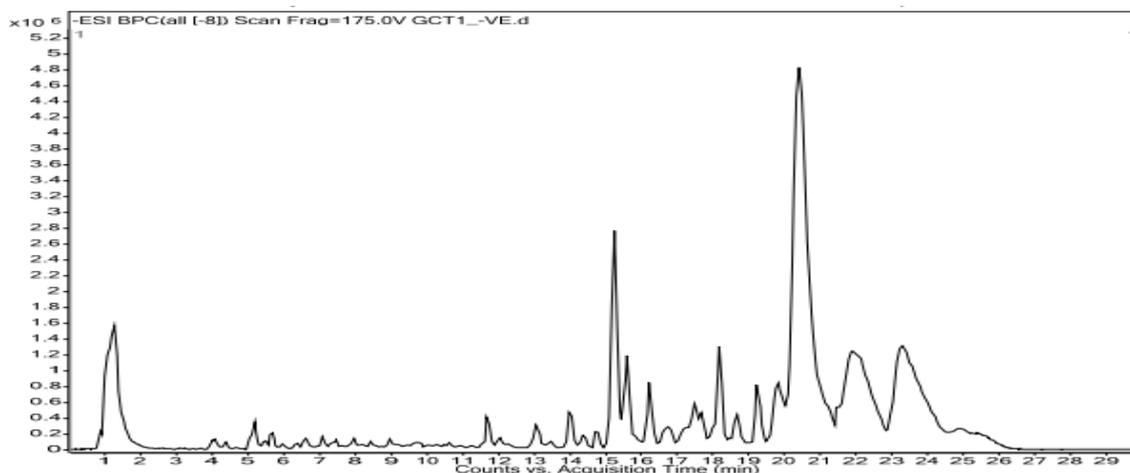


Fig 2.1: Positive chromatogram of *Padina tetrastromatica*

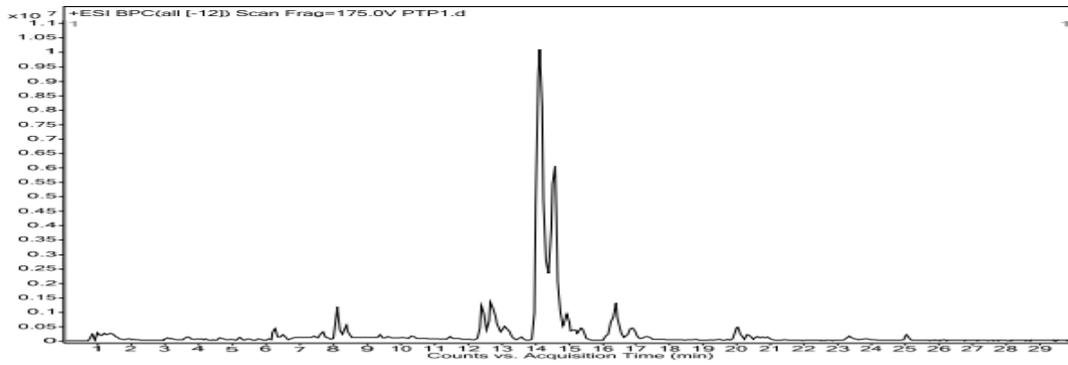


Fig 2.2: Negative chromatogram of *Padina tetrastromatica*

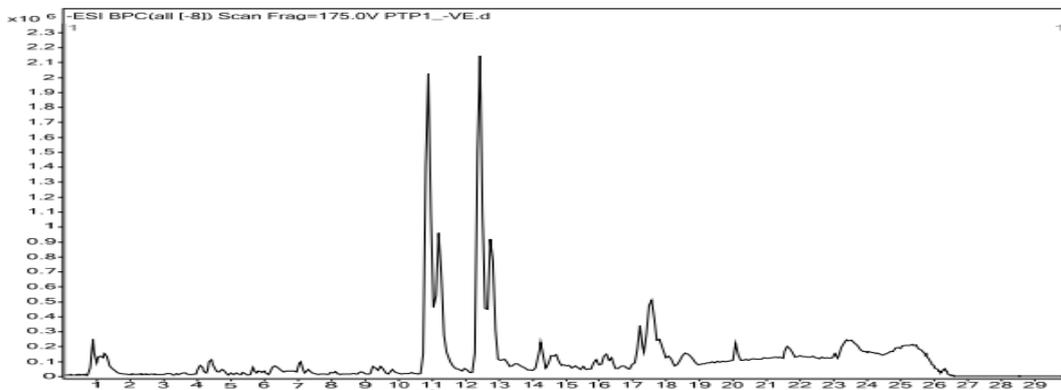


Fig 3.1: Positive chromatogram of *Caulerpa peltata*

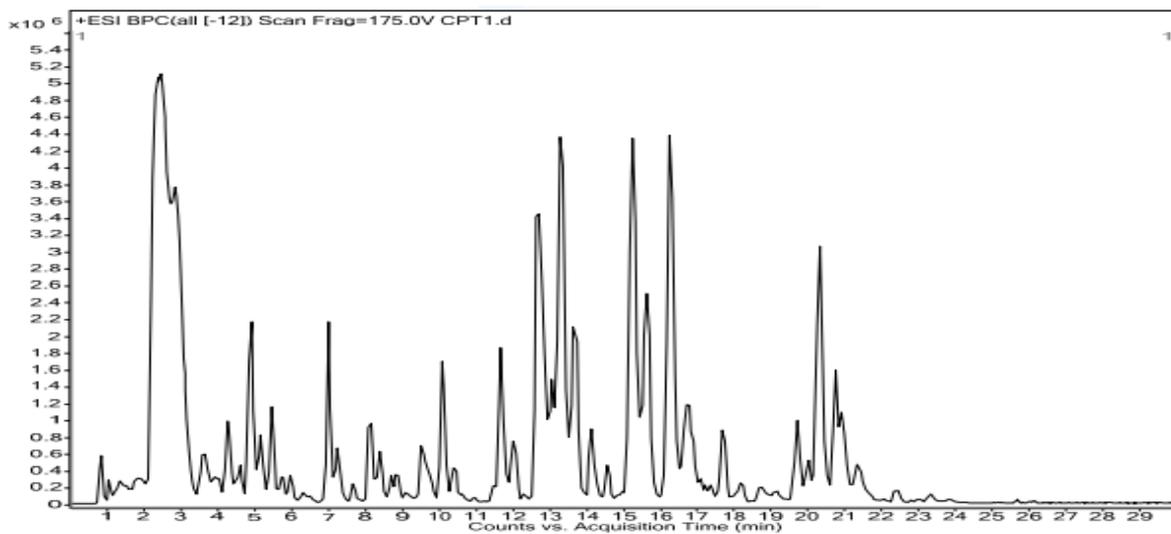


Fig 3.2: Negative chromatogram of *Caulerpa peltate*

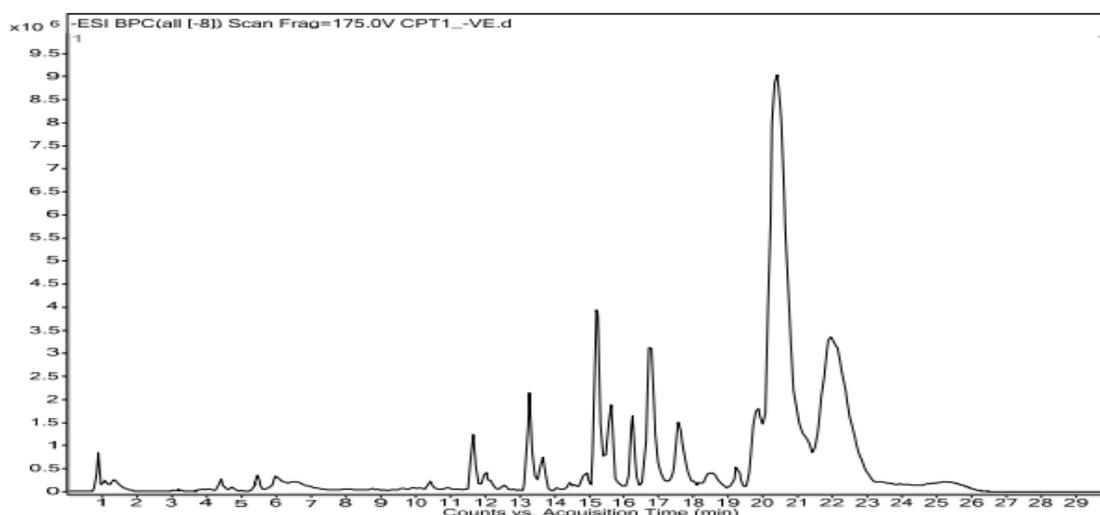


Table 5.1: Compounds present in positive chromatogram of *Gracilaria corticate*.

Sl. No.	Chemical Name	Formula	RT	Mass	Abundance
1	3beta,6beta-Dihydroxynortropane	C7 H13 N O2	1.11	143.094	174519
2	Capillene	C12 H10	2.283	154.0791	10615
3	Isocarbostyryl	C9 H7 N O	3.659	145.0517	61822
4	Feruloylputrescine	C14 H20 N2 O3	4.159	264.145	23792
5	Physovenine	C14 H18 N2 O3	7.266	262.1321	12960
6	Annofoline	C16 H25 N O2	7.307	263.1853	14343
7	Bornyl butyrate	C14 H24 O2	7.375	224.1786	12038
8	Ismine	C15 H15 N O3	8.773	257.1055	16895
9	Lactapiperanol C	C16 H26 O4	8.867	282.1799	11421
10	Fagaramide	C14 H17 N O3	9.78	247.1213	31658
11	Ethylene brassylate	C15 H26 O4	10.053	270.1826	10805
12	3-Methylbutyl methylpropanoate	C9 H18 O2	10.459	158.1315	33763
13	Vulgarone A	C15 H22 O	10.482	218.1659	23896
14	L-Menthyl acetoacetate	C14 H24 O3	10.811	240.1728	19265

Table 5.2: Compounds present in negative chromatogram of *Gracilaria corticata*

SL. NO.	Chemical Name	Formula	RT	Mass	Abundance
1	Cryogenine	C <sub>26</sub> H <sub>29</sub> N O <sub>5</sub>	10.219	435.2098	22684
2	Physapubenolide	C <sub>30</sub> H <sub>40</sub> O <sub>8</sub>	16.621	528.2717	113083
3	14,19-Dihydroaspidospermatine	C <sub>21</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub>	23.447	340.2155	1178656

 Table 6.1: Compounds present in positive chromatogram of *Padina tetrastromatica*

Sl. No.	Chemical Name	Formula	RT	Mass	Abundance
1	Pirbuterol	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub>	4.61	240.148	16210
2	Valyl-Tyrosine	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	7.092	280.1415	31243
3	Avenanthramide L	C <sub>18</sub> H <sub>15</sub> N O <sub>5</sub>	12.293	325.0972	94467
4	Hulupone	C <sub>20</sub> H <sub>28</sub> O <sub>4</sub>	11.481	332.1991	82918
5	3-(2-Methylpropanoyloxy)-8-(2-methylbutanoyloxy)-9,10-epoxy-p-mentha-1,3,5-triene	C <sub>19</sub> H <sub>26</sub> O <sub>5</sub>	11.441	334.1786	121107
6	Zanthobisquinolone	C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	12.632	362.1268	304760

 Table 6.2: Compounds present in negative chromatogram of *Padina tetrastromatica*

Sl. No.	Chemical Name	Formula	RT	Mass	Abundance
1	Zeylenol	C <sub>21</sub> H <sub>20</sub> O <sub>7</sub>	13	384.1189	190936

 Table 7.1: Compounds present in positive chromatogram of *Caulerpa peltata*

Sl. No.	Name	Formula	RT	Mass	Abundance
1	8-Hydroxy-2-chlorodibenzofuran	C <sub>12</sub> H <sub>7</sub> Cl O <sub>2</sub>	0.807	218.0139	344802

2	Isocarbostyryl	C <sub>9</sub> H <sub>7</sub> N O	3.82	145.0523	361265
3	Butyl 2-aminobenzoate	C <sub>11</sub> H <sub>15</sub> N O <sub>2</sub>	4.707	193.1094	404226
4	Bopindolol	C <sub>23</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub>	11.924	380.2111	563672
5	Lilaline	C <sub>20</sub> H <sub>17</sub> N O <sub>7</sub>	12.371	383.1024	33990
6	Hulupone	C <sub>20</sub> H <sub>28</sub> O <sub>4</sub>	14.365	332.1998	119465
7	5'-(3',4'-dihydroxyphenyl)-gamma-valerolactone glucuronide	C <sub>18</sub> H <sub>22</sub> O <sub>10</sub>	14.522	398.1237	51576
8	Caulerpin	C <sub>24</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	14.712	398.1245	83833
9	Tubulosine	C <sub>29</sub> H <sub>37</sub> N <sub>3</sub> O <sub>3</sub>	16.204	475.2814	199396
10	6-Hydroxysandoricin	C <sub>31</sub> H <sub>40</sub> O <sub>12</sub>	17.985	604.2526	59959
11	27-Nor-5b-cholestane-3a,7a,12a,24,25-pentol	C <sub>26</sub> H <sub>46</sub> O <sub>5</sub>	18.001	438.331	45825

 Table 7.2: Compounds present in negative chromatogram of *Caulerpa peltata*

Sl. No.	Chemical Name	Formula	RT	Mass	Abundance
1	Physapubenolide	C <sub>30</sub> H <sub>40</sub> O <sub>8</sub>	16.282	528.272	215273
2	Dehydrocarpaine II	C <sub>28</sub> H <sub>46</sub> N <sub>2</sub> O <sub>4</sub>	17.792	474.3454	267820
3	14,19-Dihydroaspidospermatine	C <sub>21</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub>	23.4	340.215	179483

#### 4. Discussion

Various phytochemicals were found to be exhibited by red alga *Gracilaria corticata* in the research. The initial findings of the study demonstrated resemblances to the investigations conducted by Sornalakshmi et al. 2021 and Geetha Devi and Sreedevi 2022. The *Padina tetrastrumatica* brown alga contains seven different phytochemicals in varying levels across three distinct extracts. The findings of the research aligned closely with those of Amitha et al 2023 and Johnson et al. 2014. The existence of various phytoconstituents was revealed by the qualitative phytochemical analysis of green alga *Caulerpa peltata*. The findings were similar to those of the research conducted by Sruthy and Chitra 2019.

The phenolic content in *Gracilaria corticata* was higher than the study of Sharma and John, 2022. The level of flavonoids in *Gracilaria corticata* in the research was higher than the study of Sumayya et al. 2018. The tannin levels in *Gracilaria corticata*, *Padina tetrastrumatica* and *Caulerpa peltata* were comparable to those found in the research conducted by Petchidurai et al. 2019 and Vimala et al. 2015. It was also discovered that these three types of algae have high levels of tannins. A recent study revealed

that researchers discovered a consistent glycoside content pattern in all three types of algae examined. According to Ozcan Konur (2020), seaweed contains glycosides and has bioactive effects.

The current study found that red alga *Gracilaria corticata* showed the highest antioxidant activity and green algae showed the lowest. The current investigation was found similar to the result of Narasimhan et al. 2013.

The water extract of *Gracilaria corticata* exhibited a high level of effectiveness against bacteria. The study findings matched those of Johnsi et al. 2011 in Mullur, Muttom and Kanyakumari coastline, showing comparable results in terms of mean zone of inhibition. The activity ranges of aqueous extract of *Padina tetrastromatica* also matched those in the study of Johnsi et al. 2011. The research was associated with the research on *Caulerpa peltata* by Padmakumar and Ayyakannu in 1997 and other *Caulerpa species* by Kolanjinathan in 2013.

The red alga *Gracilaria corticata* contains various bioactive compounds with significant pharmacological potential. For example, Athira et al. (2022) identified the alkaloid 3beta,6beta-Dihydroxynortropane, which may help treat cardiovascular diseases. Capillene, a polyacetylene compound shows activity against diabetes, tumors, inflammation and microbes (Servi, 2021), while the alkaloid Isocarbostryl is reported to have anticancer properties (Ingrassia, 2008). Feruloylputrescine supports heart health by acting on specific enzymes without affecting gut bacteria (Lee et al., 2024)

Other compounds include Physovenine, an alkaloid (NCBI, 2024) and annofoline, a sesquiterpenoid (Kendre et al., 2024). Bornyl butyrate serves as a flavoring with antimicrobial effects (NCBI, 2024; Caprari, 2021), while Ismine offers neuroprotective, antibacterial, antifungal and cytotoxic properties (Guo et al., 2017). Lactapiperanol C has antidiabetic effects (Borkar et al., 2023), and Fagaramide exhibits antibacterial and antifungal activity (Nna et al., 2019 and Neal 1989). Ethylene brassylate and 3-methylbutyl 2-methylpropanoate act as fragrance and flavor enhancers respectively (Api et al., 2016; NCBI, 2024). Monoterpenoids like Vulgarone A and L-menthyl acetoacetate display anti-inflammatory and multiple therapeutic properties (Parvin, 2009) and Cryogenine has anti-inflammatory effects (Rumella et al., 2008). Finally, physapubenolide, a withanolide, shows cytotoxic activity against cancer cells (Wang Hy et al., 2022).

The brown alga *Padina tetrastromatica* contains bioactive compounds with valuable pharmacological and cosmetic benefits. Pirbuterol, identified as a cosmetic ingredient, is noted for its utility in skin care (Kalasariya et al., 2023). Valyl-tyrosine has antihypertensive properties (Lafarga et al., 2020), and Avenanthramide L, a phenolic alkaloid, exhibits anti-inflammatory, anti-itching, immunomodulatory and antiproliferative effects beneficial in cancer prevention (Dvořáček, 2021 and Pretorius 2022). Hulupone provides antioxidant and anti-inflammatory benefits (Wu et al., 2020), while 3-(2-Methylpropanoyloxy)-8-(2-methylbutanoyloxy)-9,10-epoxy-p-mentha-1,3,5-triene is classified as a phenol ester (FDB010657). Zanthobisquinolone and zeylenol show antibacterial activity as reported by Nhiem et al. 2021 and Tang et al. 2014 respectively.

The green alga *Caulerpa peltata* contains numerous bioactive compounds with key medicinal benefits. 8-Hydroxy-2-chlorodibenzofuran has antimicrobial properties (Khodarahmi et al., 2015) and isocarbostryl is recognized as an anticancer agent (Ingrassia et al., 2008). Butyl 2-aminobenzoate serves as both a flavoring agent and insect repellent, while bopindolol acts as a beta-blocker (NCBI, 2024). Lilaline aids

in treating burns, ulcers, inflammation and wound healing (NCBI, 2024). Additionally, the phenolic metabolite 5'-(3',4'-dihydroxyphenyl)-gamma-valerolactone glucuronide displays notable metabolic functions (Phytohub - PHUB001754). The alkaloid Caulerpin has anti-inflammatory effects (Bhuvaneshwari & Thirumalaivasan, 2017; Ghaliaoui et al., 2024), while tubulosine is effective for fever management (Brauchli, 1964). Terpenoid 6-Hydroxysandoricin has anti-feedant qualities and 27-nor-5b-cholestane-3a,7a,12a,24,25-pentol is a significant steroid (NCBI, 2024). Physapubenolide is cytotoxic to cancer cells (Wang et al., 2022) and both Dehydrocarpaine II and 14,19-dihydroaspidospermatine demonstrate potent antioxidant effects (Tang, 1978; Saleem et al., 2020).

## 5. Conclusion

Red, brown and green algae like *Gracilaria corticata*, *Padina tetrastromatica* and *Caulerpa peltata* contain powerful bioactive phytoconstituents that display strong antioxidant and antimicrobial properties making them valuable for medicinal purposes. Chemicals such as 3beta,6beta-Dihydroxynortropine (benefits heart health), Isocarbostyryl (fights cancer), Capillene (helps with diabetes, inflammation and fighting microbes) and Physapubenolide (kills cancer cells) demonstrate the medicinal abilities of *Gracilaria corticata*. *Padina tetrastromatica* has Avenanthramide L, showing anti-inflammatory, anti-itch and cancer-fighting qualities and Pirbuterol, promoting skin well-being. *Caulerpa peltata* provides Caulerpin for anti-inflammatory effects and Lilaline for promoting wound healing in addition to 8-Hydroxy-2-chlorodibenzofuran for antimicrobial properties. The wide range of bioactive compounds in marine algae show the great possibilities of using them as natural sources of therapeutic substances for pharmaceuticals, cosmetics and functional foods. The fact that these compounds from marine macroalgae have both antioxidant and antimicrobial properties highlights their importance as valuable sources for creating natural, health-enhancing products used in pharmaceuticals, cosmetics and functional foods.

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