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# Comparative Evaluation of Phytochemical, Biochemical and Antioxidant Profiles in *Padina tetrastromatica* Hauck *and Gracilaria corticata.* J. Ag.

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**Abstract:** ***Current study evaluates the hydrographic features of seawater and compares the pigment composition, biochemical profile, phytochemical constituents and antioxidant activity of Padina tetrastromatica Hauck and Gracilaria corticata J. Ag. Seawater parameters remained within typical marine ranges, with pH 8.15 ± 0.05, dissolved oxygen 3.87 ± 0.098 mg/L and moderate nutrient levels. Pigment analyses showed higher chlorophyll fractions in P. tetrastromatica while G. corticata displayed markedly elevated carotenoid content. Biochemical assessment revealed greater protein and carbohydrate levels in G. corticata. Phytochemical screening indicated abundant flavonoids, phenols, tannins and terpenoids in extracts of both species with solvent dependent variation. Total phenolic and flavonoid content were highest in acetone extracts of P. tetrastromatica and distilled water extracts of G. corticata. DPPH assays demonstrated dose dependent antioxidant activity with acetone extracts exhibiting the strongest radical scavenging effect. Overall, both seaweeds show significant biochemical richness and antioxidant potential supporting their relevance for nutraceutical applications.***

**Keywords:** Hydrographic features, Pigment composition, Biochemical profile, Phytochemical constituents and Antioxidant activity

## 1. Introduction

Marine algae represent a diverse group of photosynthetic plant like eukaryotes that dominate marine ecosystems covering over 70% of Earth's surface. They occur as micro and macro forms and are traditionally categorized into Chlorophyceae, Phaeophyceae and Rhodophyceae based on pigmentation (Daisy et al., 2016). Although they lack true vascular structures their morphology includes holdfasts, stipes and blades enabling survival in harsh marine environments (Kuda et al., 2005). Owing to their photosynthetic pigments and adaptive features algae serve as major primary producers and key ecological components influencing nutrient cycling, habitat structure and environmental monitoring including phenomena such as eutrophication driven algal blooms. Historically, seaweeds have been used for food, medicine and industrial applications as documented in early Chinese records (Abbott, 1996) and continue to support global industries through production of agar, alginate, carrageenan and other commercial products (Mishra et al., 1993).

Nutritionally and biochemically marine macroalgae contain high levels of proteins, lipids, carbohydrates, polysaccharides, pigments and essential fatty acids with variations influenced by geography and season (Mohammed Fayaz et al., 2005; Ganesan et al., 2008). Rhodophytes in particular contribute significantly to marine secondary metabolites and serve as a dietary protein source (Teixeiera, 2013). Seaweed proteins encompass all essential amino acids (Fleurence, 1999), while lipids function in membrane structure and metabolic regulation. Carbohydrates and structural polysaccharides provide both energy and industrial value. Algal metabolites also exhibit substantial bioactivity especially antioxidant, antibacterial, antifungal and anti-inflammatory effects (Venugopal, 2009; Lee et al., 2004). Antioxidant constituents such as polyphenols, carotenoids and polysaccharides help mitigate oxidative stress linked to chronic diseases (Morgan et al., 1980; Cabrita et al., 2010). These health promoting compounds reinforce the longstanding nutritional and pharmacological relevance of seaweeds.

Marine algae further contain diverse phytochemicals including alkaloids, steroids, glycosides, flavonoids, tannins, terpenoids and saponins each contributing to various therapeutic and ecological roles (Savithramma et al., 2011). Many of these compounds demonstrate antimicrobial, anticancer, antioxidant and metabolic regulatory activities with mechanisms spanning free-radical scavenging, enzyme modulation and chemical defense (Kahkonen et al., 1999; Bohlmann & Keeling, 2008). Their wide application ranges from pharmaceuticals and cosmetics to functional foods and biofuels (Indegaard & Ostgaard, 1991). Given this extensive biochemical potential the present study aims to evaluate the nutritional and bioactive properties of two seaweed species—*Padina tetrastromatica* Hauck and *Gracilaria corticata* J. Ag. collected from Thirumullavaram Beach, Kollam, Kerala through biochemical, phytochemical, physicochemical and antioxidant analyses.

## 2. Materials and Methods

### Study Area

Seaweed samples of *Gracilaria corticata* J. Ag. and *Padina tetrastromatica* Hauck were collected from the rocky coastal region of Thirumullavaram, Kollam (8.89320°N, 76.61410°E), an area characterized by rich algal diversity and minimal freshwater inflow.

**Collection of Seaweed**
Fresh materials of *Gracilaria corticata* J. Ag. and *Padina tetrastromatica* Hauck were collected on 19 April 2024 from Thirumullavaram Beach. After collection, the specimens were thoroughly cleaned using seawater and subsequently with tap water to remove sediments and epiphytic organisms.

**Preparation of Seaweed Powder**
The cleaned samples were air-dried and later oven-dried at 40°C, powdered and stored in airtight containers for further biochemical and phytochemical analyses.

**Physico-Chemical Analysis of Seawater**
Seawater was analyzed for pH, dissolved oxygen, salinity, nitrate and phosphate using standard protocols. Nitrate concentration was measured according to Strickland and Parsons (1972) while phosphate estimation followed the method of Murphy and Riley (1962).

**Biochemical Composition of Seaweeds**
Biochemical composition of the dried seaweed powder was determined through established procedures: proteins by the Lowry method (Lowry et al., 1951), carbohydrates by the phenol–sulphuric acid assay (Dubois et al., 1956), lipids using the Bligh and Dyer extraction method (Bligh & Dyer, 1959) and chlorophyll and carotenoids following Arnon (1949) and Kirk & Allen (1965) respectively.

**Preparation of Solvent Extracts**
For phytochemical and antioxidant studies, 50 g of powdered seaweed was extracted separately with distilled water, acetone and chloroform, filtered and evaporated at 40°C before storage.

**Qualitative and Quantitative Phytochemical Screening**
Phytochemical constituents were screened following Harborne (1998). Total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu assay (Singleton & Rossi, 1965) and the colorimetric method of Chang et al. (2002).

**Antioxidant Assay**
Antioxidant capacity was evaluated using the DPPH radical scavenging assay as described by Yen and Chen (1995).

## 3. Results and Discussion

The present investigation evaluated the physicochemical characteristics of seawater from Thirumullavaram and the biochemical, phytochemical and antioxidant properties of *Padina tetrastromatica* Hauck and *Gracilaria corticata* J. Ag. The environmental parameters of the study area showed conditions favourable for the growth of intertidal macroalgae (Breeman, 1998). (Table. 1)Atmospheric and surface water temperatures exhibited only marginal variation, recording 37°C and 36°C, respectively. Seawater pH remained slightly alkaline (8.15 ± 0.05), while salinity was moderate (32.64 ± 0.07‰). Dissolved oxygen (3.87 ± 0.098 mg/L) was within the range generally supported by photosynthetic activity and atmospheric diffusion (Kamer & Stein, 2003). Nutrient levels revealed comparatively low nitrate (0.49 ± 0.015 µg at/L) and relatively higher phosphate concentration (2.91 ± 0.13 µg at/L), indicating typical oligotrophic surface-water conditions where biological uptake strongly regulates nutrient availability (Lapointe & Tenore, 1981; Dawes, 1998).

Biochemical profiling revealed that both seaweeds possessed essential nutritional constituents. Protein content was higher in *G. corticata* (17.92 ± 0.29%) than in *P. tetrastromatica* (14.36 ± 0.55%), supporting earlier findings on the comparatively rich protein status of red algae (Vinoj Kumar & Kaladharan, 2007). (Table. 3) Carbohydrates also followed the same trend being greater in *G. corticata* (7.48 ± 0.04%) than in *P. tetrastromatica* (5.03 ± 0.06%), corroborating reports of species-specific carbohydrate accumulation in marine algae (Seedevi et al., 2017). Lipid content occurred only in trace amounts in both species (0.33 ± 0.08% in *P. tetrastromatica*; 0.43 ± 0.06% in *G. corticata*), which is typical of most tropical seaweeds (Mashaghi et al., 2013).

Pigment analysis showed marked variation between the species. *P. tetrastromatica* exhibited higher concentrations of total chlorophyll (0.0361 ± 0.0095 mg/g) compared to *G. corticata* (0.0061 ± 0.0021 mg/g). In contrast, carotenoid levels were considerably higher in *G. corticata* (2.7538 ± 0.2943 mg/g) than in *P. tetrastromatica* (0.0420 ± 0.0139 mg/g) indicating differential photoprotective strategies and light-harvesting efficiencies in brown and red algae (Table. 2).

Phytochemical screening of both species demonstrated abundant presence of major bioactive groups—flavonoids, phenols, tannins, terpenoids, saponins, coumarins and cardiac glycosides with clear solvent dependent variation, consistent with solubility and polarity effects reported earlier (Hediat et al., 2010). Acetone and distilled water extracts of both algae consistently yielded more phytochemicals than chloroform, in agreement with observations that polar solvents extract greater quantities of polyphenolic constituents (Shyamala et al., 2014). Alkaloids were absent in several extracts, while steroids were detected only in selective extracts of *P. tetrastromatica*. Terpenoids were found across almost all solvent types reflecting their broad solubility profile and metabolic importance (Table. 4 & 5) (Bohlmann & Keeling, 2008).

Quantitative phenolic and flavonoid profiling further highlighted these solvent effects. In *P. tetrastromatica*, the highest total phenolic (8.89 ± 0.13 mg/g) and flavonoid (19.88 ± 0.19 mg/g) levels were recorded in acetone extracts followed by distilled water (4.60 ± 0.09 mg/g phenols; 18.78 ± 0.22 mg/g flavonoids) and chloroform (2.68 ± 0.25 mg/g phenols; 15.68 ± 0.16 mg/g flavonoids). Conversely, *G. corticata* showed maximum phenolic (9.71 ± 0.19 mg/g) and flavonoid (18.75 ± 0.17 mg/g) content in distilled water extracts, followed by acetone (7.86 ± 0.14 mg/g; 15.82 ± 0.15 mg/g) and chloroform (1.32 ± 0.21 mg/g; 7.97 ± 0.14 mg/g). (Table. 6) The dominance of polyphenols in polar extracts aligns with reports that phenolic compounds show greater solubility in hydrophilic solvents (Waterman & Mole, 1994) and that brown algae generally possess high phenolic reserves (Chkhikvishvili & Ramazanav, 2000). These polyphenols are known contributors to antioxidant function due to their reducing and metal chelating abilities (Manach et al., 2004).

Antioxidant activity assessed through DPPH radical scavenging revealed a concentration dependent increase in all extracts of both algae. In *P. tetrastromatica*, acetone extract exhibited the highest scavenging activity (60.71 ± 0.29% at 500 µg/mL) followed by distilled water (51.61 ± 0.53%) and chloroform (34.63 ± 0.42%). In *G. corticata*, the chloroform extract showed the maximum activity (49.69 ± 0.24%) followed by acetone (40.66 ± 0.45%) and distilled water (38.55 ± 0.40%) (Table. 7, 8, 9 & 10). The strong antioxidant efficiency of extracts rich in phenolics supports the established correlation between polyphenol content and free-radical quenching ability (Rice-Evans et al., 1997; Nurul et al., 2011). Similar antioxidant potential in *G. corticata* has also been reported by Kumar et al. 2010, who attributed the activity to its phenolic constituents.

**Table 1:** Physico-chemical analysis of sea water

| Temperature ($^0$C) | | pH | Salinity (‰) | Dissolved oxygen (mg/l) | Nutrients (µg at/l) | |
|---|---|---|---|---|---|---|
| At. | S.W. | 8.15 ± 0.05 | 32.64 ± 0.07 | 3.87 ± 0.098 | Nitrate | Phosphate |
| 370C | 36$^0$C | | | | 0.49 ± 0.015 | 2.91 ± 0.13 |

At. – Atmosphere, S.W- Surface water,

**Table 2:** Photosynthetic pigments of two seaweed species (mg/g dry wt.)

| Parameters | *Padina tetrastromatica* Hauck (mg/g dry wt) | *Gracilaria corticate* J.Ag. (mg/g dry wt) |
|---|---|---|
| Chlorophyll a | 0.0156 ± 0.0096 | 0.0027 ± 0.0010 |
| Chlorophyll b | 0.0109 ± 0.0096 | 0.0024 ± 0.0011 |
| Total Chlorophyll | 0.0361 ± 0.0095 | 0.0061 ± 0.0021 |
| Carotenoids | 0.0420 ± 0.0139 | 2.7538 ± 0.2943 |

**Table 3:** Biochemical composition of seaweeds

| Parameters | *Padina tetrastromatica* Hauck (%) | *Gracilaria corticate* J. Ag (%) |
|---|---|---|
| Protein | 14.36 ± 0.55 | 17.92 ± 0.29 |
| Carbohydrate | 5.03 ± 0.06 | 7.48 ± 0.04 |
| Lipid | 0.33 ± 0.08 | 0.43 ± 0.06 |

**Table 4:** Phytochemical constituents of various extracts of *Padina tetrastromatica* Hauck.

| Phytochemicals | Solvents | | |
|---|---|---|---|
| | Distilled Water | Acetone | Chloroform |
| Alkaloid | - | +++ | - |
| Steroid | - | +++ | ++ |
| Flavonoid | +++ | +++ | +++ |
| Phenol | +++ | +++ | + |
| Coumarin | +++ | +++ | - |
| Cardiac glycoside | ++ | +++ | - |
| Tannins | +++ | +++ | - |
| Terpenoids | +++ | +++ | +++ |
| Saponin | +++ | - | - |

(+) – Present, (++) – Moderate,
(+++) – Abundant, (-) – Absent

**Table 5:** Phytochemical constituents of various extracts of *Gracilaria corticata* J.Ag.

| Phytochemicals | Solvents | | |
|---|---|---|---|
| | Distilled Water | Acetone | Chloroform |
| Alkaloid | - | + | ++ |
| Steroid | - | - | - |
| Flavonoid | ++ | +++ | +++ |
| Phenol | ++ | +++ | + |
| Coumarin | +++ | ++ | - |
| Cardiac glycoside | +++ | + | + |
| Tannins | +++ | + | - |
| Terpenoids | +++ | - | +++ |
| Saponins | +++ | - | - |

(+) – Present, (++) – Moderate,
(+++) – Abundant, (-) – Absent

**Table 6:** Total phenolic and flavonoid content of various extracts of *Padina tetrastromatica* Hauck and *Gracilaria corticata* J.Ag.

| Algae | Solvents | Phenol (mg/g) | Flavonoid (mg/g) |
|---|---|---|---|
| *Padina tetrastromatica* | Distilled water | 4.60 ± 0.09 | 18.78 ± 0.22 |
| | Acetone | 8.89 ± 0.13 | 19.88 ± 0.19 |
| | Chloroform | 2.68 ± 0.258 | 15.68 ± 0.16 |
| *Gracilaria corticata* | Distilled water | 9.71 ± 0.19 | 18.75 ± 0.17 |
| | Acetone | 7.86 ± 0.14 | 15.82 ± 0.15 |
| | Chloroform | 1.32 ± 0.21 | 7.97 ± 0.14 |

**Table 7:** DPPH free radical scavenging activity of distilled water seaweed extracts

| S. No | Concentration (µg/ml) | % Activity ± SD | | |
|---|---|---|---|---|
| | | Standard (Ascorbic acid) | *Padina tetrastromatica* Hauck | *Gracilaria corticata* J.Ag |
| 1 | 100 | 66.66 ± 0.57 | 42.78 ± 0.21 | 30.64 ± 0.44 |
| 2 | 200 | 65.59 ± 0.52 | 45.62 ± 0.54 | 33.70 ± 0.47 |
| 3 | 300 | 67.67 ± 2.08 | 47.44 ± 0.45 | 34.49 ± 0.45 |
| 4 | 400 | 71.66 ± 0.57 | 49.33 ± 0.47 | 36.57 ± 0.42 |
| 5 | 500 | 73.62 ± 0.54 | 51.61 ± 0.53 | 38.55 ± 0.40 |

**Table 8:** DPPH free radical scavenging activity of acetone seaweed extracts

| S No | Concentration (µg/ml) | % Activity ± SD | | |
|---|---|---|---|---|
| | | Standard (Ascorbic Acid) | *Padina tetrastromatica* Hauck | *Gracilaria corticata* J.Ag |
| 1 | 100 | 70.5 ± 0.70 | 50.11 ± 0.47 | 32.18 ± 0.43 |
| 2 | 200 | 74.21 ± 0.70 | 52.60 ± 0.52 | 34.50 ± 0.43 |
| 3 | 300 | 76.42 ± 0.77 | 55.54 ± 0.50 | 36.52 ± 0.44 |
| 4 | 400 | 77.28 ± 0.64 | 57.14 ± 1.01 | 38.52 ± 0.41 |
| 5 | 500 | 78.20 ± 0.05 | 60.71 ± 0.29 | 40.66 ± 0.45 |

**Table 9:** DPPH free radical scavenging activity of chloroform seeweed extracts

| S No. | Concentration (µg/ml) | % Activity (± SD) | | |
|---|---|---|---|---|
| | | Standard (Ascorbic acid) | *Padina tetrastromatica* Hauck | *Gracilaria corticata* J.Ag |
| 1 | 100 | 53.33 ± 0.46 | 27.81 ± 0.15 | 42.26 ± 0.45 |
| 2 | 200 | 52.32 ± 0.57 | 28.70 ± 0.30 | 43.49± 0.44 |
| 3 | 300 | 55.48 ± 0.48 | 29.60 ± 0.44 | 44.50 ± 0.44 |
| 4 | 400 | 56.42 ± 0.51 | 32.67 ± 0.45 | 46.07 ± 0.89 |
| 5 | 500 | 58.47 ±0.45 | 34.63 ± 0.42 | 49.69 ± 0.24 |

**Table 10:** ANOVA Results for Species × Concentration in Three Solvents

| Solvent | Factor | F - Value | P - Value | Significance |
|---|---|---|---|---|
| Distilled Water | Species | 5683.50 | $4.93 \times 10^{-30}$ | Highly significant |
| | Concentration | 266.77 | $1.63 \times 10^{-19}$ | Highly significant |
| Acetone | Species | 9310.77 | $1.34 \times 10^{-32}$ | Highly significant |
| | Concentration | 298.80 | $4.32 \times 10^{-20}$ | Highly significant |
| Chloroform | Species | 4205.66 | $1.80 \times 10^{-28}$ | Highly significant |
| | Concentration | 129.51 | $7.08 \times 10^{-16}$ | Highly significant |

Two-way ANOVA revealed that in both the species, the inferred concentration had a highly significant effect ($p < 0.001$) on the DPPH free radical scavenging activity of the seaweed extracts across all solvents tested. *Padina tetrastromatica* consistently exhibited significantly higher antioxidant activity compared to *Gracilaria corticata* in distilled water and acetone extracts, while chloroform extracts also showed strong species differences, though with relatively lower activity overall. Increasing concentration from 100–500 µg/ml produced a significant enhancement in antioxidant activity in all solvent systems. These results confirm that antioxidant potential varies markedly with **species**, **solvent polarity**, and **extract concentration**.

## 4. Conclusion

The present investigation demonstrates that *Padina tetrastromatica* and *Gracilaria corticata* possess rich biochemical, phytochemical and antioxidant profiles, highlighting their value as potential sources of bioactive compounds. Both seaweeds contained appreciable amounts of proteins, carbohydrates, lipids, photosynthetic pigments, phenols and flavonoids though their levels varied across solvent extracts. The phytochemical screening confirmed the presence of key metabolites such as terpenoids, tannins, coumarins, saponins and cardiac glycosides supporting their therapeutic relevance. Antioxidant assays revealed that the radical-scavenging activity increased with extract concentration with *Padina* showing stronger activity in acetone extracts and *Gracilaria* in chloroform extracts. Overall, the findings highlight that both species hold significant nutraceutical and pharmaceutical potential and each exhibits distinct chemical strengths rather than one being superior to the other.

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