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Comparative evaluation of total phenolic content in selected marine macroalgae from Narara Bet (Gulf of Kachchh, India): Influence of species and preservative methods

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Abstract
Marine macroalgae are recognized as rich sources of bioactive compounds, particularly phenolic substances with significant antioxidant potential. The present study aimed to evaluate the total phenolic content in selected marine macroalgae from Narara Bet, a prominent intertidal region of the Gulf of Kachchh, India, and to assess the influence of species variation and preservation methods on phenolic estimation. A total of 26 macroalgal species belonging to Chlorophyta, Phaeophyta, and Rhodophyta were collected. Total phenolic content was determined using Bray & Thorpe's method and expressed as mg/g of algal material. Considerable variation in phenolic content was observed among species, with the highest values recorded in *Acanthophora spicifera* (20.59 mg/g) and *Sargassum prismaticum* (18.35 mg/g), while comparatively lower values were noted in species such as *Amphiroa fragilissima* and *Solieria robusta*. Division-wise analysis indicated relatively higher phenolic accumulation in Rhodophyta. Furthermore, comparative evaluation of preservation methods in selected species revealed that oven-dried samples generally exhibited higher phenolic content, followed by fresh samples, whereas freeze-stored samples showed comparatively lower values. The findings highlight that both species selection and preservation technique significantly influence the estimation of total phenolic content in marine macroalgae. This study underscores the potential of intertidal macroalgae as promising natural sources of phenolic compounds and emphasizes the importance of appropriate processing methods for their effective utilization.

Keywords: Macroalgae, total phenols, narara bet, antioxidant potential

Introduction

Marine macroalgae, commonly known as seaweeds, constitute an ecologically and economically important component of coastal and intertidal ecosystems (Shalaby, 2011; Dhargalkar & Kavlekar, 2001) [4, 14]. They are widely recognized as rich sources of diverse bioactive compounds, including polysaccharides, proteins, lipids, vitamins, and secondary metabolites such as phenolic compounds (Holdt & Kraan, 2011; Cornish & Garbary, 2010) [3, 7]. In recent years, macroalgae have gained considerable attention due to their potential applications in food, pharmaceutical, cosmetic, and nutraceutical industries, primarily owing to their antioxidant, antimicrobial, and anti-inflammatory properties (Gupta & Abu-Ghannam, 2011; Wijesinghe & Jeon, 2012) [5, 18]. Among the various bioactive constituents, phenolic compounds are of particular interest due to their strong antioxidant activity and their role in protecting algal cells against environmental stressors such as ultraviolet radiation, salinity fluctuations, and herbivory (Ragan & Glombitza, 1986; Targett & Arnold, 2001) [12, 17]. These compounds contribute significantly to the defense mechanisms of algae and are also associated with various health-promoting effects when utilized in human applications (Li *et al*., 2011). The total phenolic content (TPC) is therefore considered an important biochemical parameter for evaluating the bioactive potential of marine macroalgae (Singleton *et al*., 1999) [15].

It is well established that the phenolic content in macroalgae varies considerably among different species and taxonomic groups, such as Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) (Stengel *et al*., 2011; Matanjun *et al*., 2008) [10, 16]. Such variations are often attributed to differences in genetic makeup, environmental conditions, and ecological adaptations (Stengel *et al*., 2011) [16]. Additionally, factors such as seasonal variation, habitat characteristics, and physiological state of the algae further influence the accumulation of phenolic compounds (Connan *et al*., 2004; Holdt & Kraan, 2011) [2, 7]. Apart from biological variation, post-harvest handling and preservation methods also play a crucial role in determining the stability and extractability of phenolic compounds (Rajauria *et al*., 2010) [11]. Techniques such as oven drying, freezing, and analysis of fresh samples may lead to differences in the measured phenolic content due to factors like moisture loss, enzymatic activity, and degradation or transformation of phenolic constituents (Rajauria *et al*., 2010; Gupta *et al*., 2011) [5, 11]. Understanding the impact of these preservation methods is essential for accurate biochemical evaluation and for optimizing the utilization of macroalgae as sources of natural antioxidants.

Narara Bet, located in the Gulf of Kachchh along the Gujarat coast of India, represents a unique intertidal ecosystem characterized by rich macroalgal diversity (Jha *et al*., 2009; Sahoo *et al*., 2001) [8, 13]. Despite its ecological significance, comprehensive studies focusing on the biochemical profiling of macroalgae from this region, particularly with respect to phenolic content and the influence of preservation methods, remain limited. In this context, the present study was undertaken to evaluate the total phenolic content in selected marine macroalgae collected from Narara Bet and to examine the influence of species variation and different preservation methods on

phenolic estimation. The findings of this study are expected to contribute to a better understanding of the biochemical potential of intertidal macroalgae and their prospective applications as natural sources of bioactive compounds.

Materials & Methods

a. Sample Collection & Storage

Macroalgal samples were collected from the intertidal zones of Narara Bet during the period September to December 2025. A total of 26 species representing different taxonomic groups were collected manually during low tide conditions. The collected samples were thoroughly washed with seawater, brought to the laboratory and washed properly with distilled water to remove sand particles, epiphytes, and other extraneous materials. The specimens were identified based on morphological characteristics using standard taxonomic keys and relevant literature. The algal samples were then subjected to different preservation methods for comparative analysis of total phenolic content and are named as Fresh samples, Freeze Cold and Oven Dried. Fresh samples were a portion of the cleaned samples that were immediately processed for biochemical analysis. Freeze cold samples were stored at low temperature in refrigerator until further analysis. For Oven drying the samples were dried in a hot air oven at a temperature of 40℃ for 7 days. The dried material was then used for further analysis.

b. Preparation of Extract

The algal samples (Fresh, Freeze Cold Stored & Oven-dried) were weighed (100 mg) and were homogenized with 80% methanol. They were centrifuged for 15 minutes at around 7000 – 9000 rpm. The supernatant was filtered and was used as extract for further estimation.

c. Total Phenolic Content

Total Phenolic Content was determined by Folin-Ciocalteau method (Bray & Thorpe, 1954) [1]. An aliquot of 1 ml of the algal extract (supernatant) was taken in a test tube. To this, 1 ml of 20% sodium carbonate (Na_2CO_3) solution was added, followed by the addition of 0.5 ml of Folin–Ciocalteu reagent (1N). The contents were mixed thoroughly, and the test tubes were stoppered. The reaction mixture was then placed in a boiling water bath for 10 minutes to allow color development. After incubation, the tubes were cooled to room temperature. The final volume of the reaction mixture was made up to 20 ml using distilled water and allowed to stand undisturbed for some time for complete color stabilization. The absorbance (optical density) was measured at 660 nm using a UV-VIS spectrophotometer. A blank was prepared simultaneously by replacing the extract with 1 ml distilled water and following the same procedure. The total phenolic content was calculated using the regression equation of tannic acid standard: $X = 400Y – 0.001$ where:

X = Total phenolic content (mg/g plant material)

Y = Absorbance at 660 nm

The results were expressed as mg phenols per gram of plant material. The sets were carried out in triplicates and the values were statistically analysed and expressed as Mean ± SE.

Results & Discussion

1. Total Phenolic Content in Macroalgae

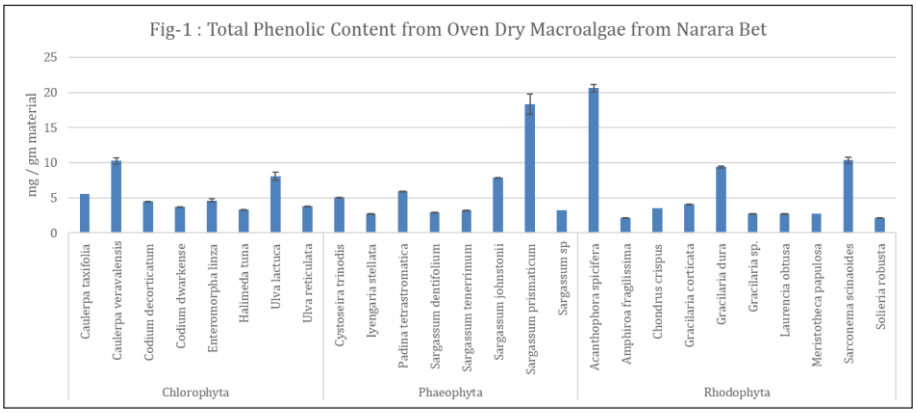
The quantitative assessment of 26 marine macroalgal species collected from the intertidal region of Narara Bet revealed significant interspecific variability in total phenolic content (TPC). As presented in Table 1, TPC values ranged from a minimum of 2.14 mg/g in *Amphiroa fragilissima* to a maximum of 20.59 mg/g in *Acanthophora spicifera*. This nearly ten-fold variation highlights the substantial metabolic diversity among macroalgae inhabiting the same ecological niche.

The exceptionally high phenolic content observed in *Acanthophora spicifera* (20.59 mg/g), followed by *Sargassum prismaticum* (18.35 mg/g), indicates the presence of strong biochemical defense mechanisms in these species. Phenolic compounds in macroalgae, including phlorotannins in brown algae and bromophenols in red algae, play a crucial role as antioxidants and protective agents against ultraviolet radiation. The elevated TPC in these species may represent an adaptive response to the harsh environmental conditions of the Narara Bet intertidal zone, where organisms are frequently exposed to intense solar radiation, desiccation, and fluctuating salinity during tidal cycles.

Table 1: Total Phenols from Oven Dry Material (100 mg)

Sr. No.	Division	Algae	From Oven Dry (mg/g)
1	Chlorophyta	*Caulerpa taxifolia*	5.5199 ± 0.0000
2		*Caulerpa veravalensis*	10.2665 ± 0.4463
3		*Codium decorticatum*	4.4532 ± 0.0108
4		*Codium dwarkense*	3.6799 ± 0.0108
5		*Enteromorpha linza*	4.5999 ± 0.2317
6		*Halimeda tuna*	3.3465 ± 0.0108
7		*Ulva lactuca*	8.0532 ± 0.5963
8		*Ulva reticulata*	3.8132 ± 0.0108
9	Phaeophyta	*Cystoseira trinodis*	5.0932 ± 0.0108
10		*Iyengaria stellata*	2.7332 ± 0.0108
11		*Padina tetrastromatica*	5.9332 ± 0.0288
12		*Sargassum dentifolium*	2.9732 ± 0.0108
13		*Sargassum tenerrimum*	3.2265 ± 0.0108
14		*Sargassum johnstonii*	7.8265 ± 0.0662
15		*Sargassum prismaticum*	18.3599 ± 1.4504
16		*Sargassum sp.*	3.1999 ± 0.0000
17	Rhodophyta	*Acanthophora spicifera*	20.5999 ± 0.5346
18		*Amphiroa fragilissima*	2.1465 ± 0.0108
19		*Chondrus crispus*	3.4799 ± 0.0000

20		*Gracilaria corticata*	4.1065 ± 0.0108
21		*Gracilaria dura*	9.3732 ± 0.1603
22		*Gracilaria sp.*	2.7199 ± 0.0188
23		*Laurencia obtusa*	2.7865 ± 0.0108
24		*Meristotheca papulosa*	2.7599 ± 0.000
25		*Sarconema scinaoides*	10.3465 ± 0.4242
26		*Solieria robusta*	2.1865 ± 0.0217



The variation in total phenolic content among different macroalgal species (Table 1; Figure 1) was found to be statistically significant ($p < 0.05$), indicating that species-specific factors play a crucial role in phenolic accumulation. The relatively low standard error values observed for most species suggest good reproducibility and reliability of the experimental data.

A comparative analysis across taxonomic divisions revealed a clear hierarchical trend in phenolic accumulation: Rhodophyta > Phaeophyta > Chlorophyta, as illustrated in Figure 2. Although several studies have identified Phaeophyta as the dominant source of phenolic compounds due to the presence of phlorotannins (Connan *et al*., 2004; Holdt & Kraan, 2011) [2, 7], the present findings highlight the significant phenolic potential of tropical Rhodophyta species from the Gulf of Kachchh. Within Rhodophyta, high phenolic values recorded in *Acanthophora spicifera* and *Sarconema scinaioides* (10.34 mg/g) are consistent with reports that red algae synthesize complex bromophenols and polyphenolic compounds to counter oxidative stress. In Phaeophyta, *Sargassum prismaticum* exhibited the highest phenolic content, further supporting earlier observations that members of the genus *Sargassum* are prolific producers of secondary metabolites. In contrast, Chlorophyta generally exhibited lower phenolic content, with species such as *Halimeda tuna* (3.34 mg/g) and *Codium dwarkense* (3.67 mg/g) showing minimal accumulation. This comparatively lower phenolic level may indicate a greater reliance on alternative antioxidant mechanisms, such as carotenoids or enzymatic defense systems, rather than phenolic-based protection.

The division-wise variation observed in the present study is in agreement with earlier reports indicating that red and brown algae tend to accumulate higher levels of phenolic compounds compared to green algae (Connan *et al*., 2004; Holdt & Kraan, 2011) [2, 7]. Such differences are often attributed to variations in metabolic pathways and ecological strategies among algal groups. Furthermore, the significant interspecific variability in TPC supports previous findings that phenolic accumulation in macroalgae is influenced by genetic factors, environmental conditions, and ecological adaptations (Stengel *et al*., 2011; Matanjun *et al*., 2008) [10, 16]. Intertidal macroalgae are frequently subjected to environmental stressors such as high irradiance, salinity fluctuations, and periodic desiccation, which are known to stimulate the synthesis of protective secondary metabolites, including phenolic compounds.

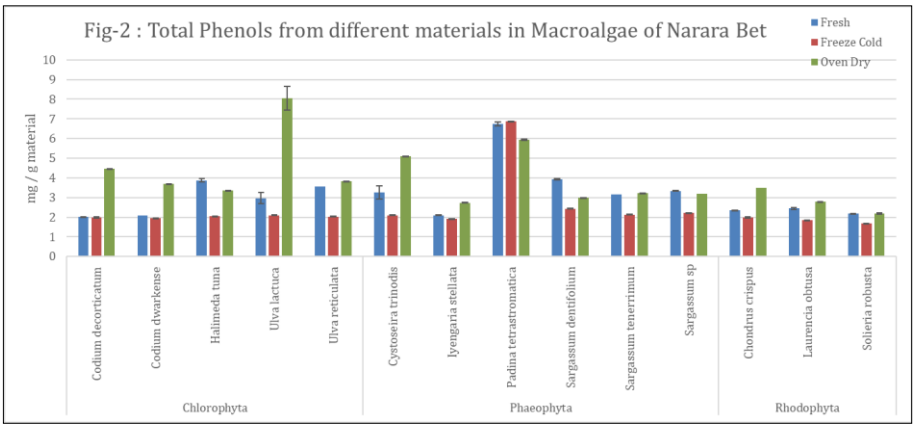
2. Effect of Preservation Methods on Phenolic Content

Due to variability in biomass availability among macroalgal species from the intertidal region of Narara Bet, the effect of preservation methods on total phenolic content (TPC) was evaluated in a subset of 14 representative species. The results, presented in Table 2 and illustrated in Figure 2, demonstrated a statistically significant influence of preservation methods on phenolic estimation ($p < 0.05$), with a consistent trend observed as: Oven-dried > Fresh > Freeze-stored samples.

Table 2: Total Phenols Content Using Different Storage Methods from Selected Macroalgae

Sr. No.	Division	Algae	From Fresh (mg/g)	From Freeze Cold (mg/g)	From Oven Dry (mg/g)
1	Chlorophyta	*Codium decorticatum*	2.0132 ± 0.0108	1.9999 ± 0.0326	4.4532 ± 0.0108
2		*Codium dwarkense*	2.0799 ± 0.000	1.9465 ± 0.0108	3.6799 ± 0.0108
3		*Halimeda tuna*	3.8665 ± 0.0850	2.0939 ± 0.0188	3.3465 ± 0.0108
4		*Ulva lactuca*	2.9599 ± 0.2828	2.0932 ± 0.0108	8.0532 ± 0.5963
5		*Ulva reticulata*	3.5599 ± 0.0000	2.0265 ± 0.0108	3.8132 ± 0.0108
6	Phaeophyta	*Cystoseira trinodis*	3.2665 ± 0.3321	2.0932 ± 0.0108	5.0932 ± 0.0108
7		*Iyengaria stellata*	2.0932 ± 0.0108	1.9065 ± 0.0108	2.7332 ± 0.0108
8		*Padina tetrastromatica*	6.7332 ± 0.1038	6.8665 ± 0.0108	5.9332 ± 0.0288
9		*Sargassum dentifolium*	3.9199 ± 0.0377	2.4265 ± 0.0108	2.9732 ± 0.0108
10		*Sargassum tenerrimum*	3.1599 ± 0.0000	2.1332 ± 0.0108	3.2265 ± 0.0108

11		*Sargassum sp.*	3.3332 ± 0.0108	2.2132 ± 0.0108	3.1999 ± 0.0000
12	Rhodophyta	*Chondrus crispus*	2.3465 ± 0.0108	1.9865 ± 0.0217	3.4799 ± 0.0000
13		*Laurencia obtusa*	2.4399 ± 0.0498	1.8532 ± 0.0108	2.7865 ± 0.0108
14		*Solieria robusta*	2.1732 ± 0.0108	1.6799 ± 0.0188	2.1865 ± 0.0217



Effect of Oven drying: Oven-dried samples consistently exhibited the highest TPC among all preservation methods. This increase may be attributed to a combination of factors. Firstly, the removal of moisture during drying results in a relative concentration of phenolic compounds per unit dry weight. Secondly, thermal treatment facilitates structural disruption of algal cell walls, enhancing solvent penetration and improving the extractability of phenolic compounds. Additionally, drying may lead to the inactivation of degradative enzymes such as polyphenol oxidase, which otherwise contribute to the oxidation and loss of phenolic compounds in fresh tissues.

These findings are in agreement with earlier reports indicating that controlled drying can enhance the measurable phenolic content due to improved extraction efficiency and reduced enzymatic degradation (Gupta *et al*., 2011) [5]. However, it is important to note that excessive heating may degrade heat-sensitive phenolic constituents, suggesting that controlled drying conditions are essential.

Effect of Freeze Cold Storage: In contrast, freeze-stored samples exhibited comparatively lower TPC values than both fresh and oven-dried samples (Table 2; Figure 2). This reduction may be attributed to structural and biochemical changes occurring during freezing and thawing processes. The formation of ice crystals during freezing can disrupt cellular integrity, leading to the release of oxidative enzymes and subsequent degradation of phenolic compounds upon thawing. Furthermore, partial oxidation or polymerization of phenolic compounds during storage may reduce their reactivity with the Folin–Ciocalteu reagent, resulting in lower spectrophotometric estimation. Similar observations have been reported in previous studies, where storage conditions influenced the stability and extractability of phenolic compounds (Rajauria *et al*., 2010) [11].

Species- Specific Response to Preservation Methods: The extent of variation in phenolic content across preservation methods was found to be species-dependent. Certain species, such as *Ulva lactuca*, exhibited a marked increase in TPC following oven drying, whereas others showed relatively minor variations across treatments. This indicates that the stability and extractability of phenolic compounds are influenced not only by external processing conditions but also by intrinsic factors such as biochemical composition and cellular structure of the algae. Such species-specific responses highlight the importance of selecting appropriate preservation techniques tailored to individual taxa for accurate biochemical evaluation.

3. Biotechnological implications

The present study highlights the significant potential of intertidal macroalgae from Narara Bet as sources of natural phenolic compounds with antioxidant properties. The pronounced interspecific variation in TPC suggests that species selection is a critical factor in the exploration and utilization of macroalgae for biotechnological applications. Species such as *Acanthophora spicifera* and *Sargassum prismaticum*, which exhibited higher phenolic content, may be considered promising candidates for further investigation in pharmaceutical, nutraceutical, and cosmeceutical industries. In addition, the study emphasizes the importance of standardizing post-harvest processing techniques, as preservation methods significantly influence the estimation and recovery of phenolic compounds. Based on the present findings, controlled oven drying appears to be a suitable method for maximizing phenolic yield in tropical marine macroalgae. Overall, the results underscore the need to align species selection with appropriate processing strategies to ensure efficient utilization of marine macroalgae as sustainable sources of bioactive compounds.

Conclusion

Total phenolic content varied widely among the studied macroalgae, with higher values recorded in certain red and brown algal species, which may be attributed to their adaptive responses to intertidal stress conditions such as high irradiance, salinity fluctuations, and desiccation. Preservation methods significantly influenced phenolic estimation, with oven drying enhancing extractability and stability of phenolic compounds, while freeze storage resulted in comparatively lower recovery.

These findings indicate that both species selection and post-harvest processing play a crucial role in determining phenolic yield. The study provides a basis for selecting suitable macroalgal species and optimizing processing methods for the efficient utilization of marine-derived phenolic compounds. Further studies focusing on isolation and characterization of specific phenolics are required to explore their potential applications in pharmaceutical and nutraceutical fields.

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