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Assessment of Total Phenolic Content and In Vitro Antioxidant Activity in Brown Seaweeds *Turbinaria conoides* and *Padina tetrastromatica* from the Mandapam Coast

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Abstract

Marine macroalgae have gained considerable scientific interest in recent years due to their noteworthy composition, which includes essential elements such as functional polyphenols, minerals, dietary fibres, proteins, polysaccharides, and vitamins, in addition to their ecological functions. This study aimed to extract these antioxidant-rich bioactive compounds from two brown seaweed species, *Turbinaria conoides* and *Padina tetrastromatica*, sourced from the Mandapam Coastal region of Tamil Nadu, India. The extraction process involved using a multi-enzyme complex consisting of a carbohydrase enzyme (Viscozyme® L) and two protease enzymes (Flavourzyme® 1000 L and Alcalase® 2.4L FG), either separately or in combination. The total phenolic content (TPC) and extract yield under different enzyme treatments were assessed, revealing notably higher values for the Alcalase® extract (73.3±1.5% yield and 3.15±0.2 mg GAE/g extract TPC). Further characterization of the extracts involved evaluating their *in vitro* antioxidant activity through total antioxidant activity (TAA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, and Ferric (Fe^{3+}) reducing antioxidant power (FRAP). The viscozyme®-extracted product displayed higher TAA (6.27±1.1 mg AAE/g extract) and DPPH scavenging activity (4.2±0.7 mg IC_{50}). However, the extract from *T. conoides* treated with the combination of enzymes showed higher FRAP (4.01±0.2 mg GAE/g extract). In conclusion, this research demonstrates the viability of using enzyme-assisted extraction methods to obtain valuable antioxidants from brown algae, paving the way for their use in the health and food industries.

Keywords: Phytochemical analysis, green chemistry extraction, marine bioactives, seaweed polyphenols, natural antioxidants

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Introduction

Seaweeds are important marine bioresources rich in biologically active phytochemicals, including fucoidan, phlorotannins, alginates, carotenoids (Kumar et al., 2020), proteins, PUFAs, polysaccharides, phenolics, secondary metabolites, pigments (Shahidi & Ambigaipalan, 2015; Hossain, Dave, & Shahidi, 2020; Ghosh et al., 2022), dietary fibers, amino acids, and vitamins (Balboa, Conde, Moure, Falqué, & Domínguez, 2013). These valuable metabolites and bioactive molecules have economic importance, prompting scientific exploration. Brown algae (Phaeophyceae) contain higher antioxidant levels compared to green (Chlorophyceae) and red (Rhodophyceae) algae (Remya et al., 2022), making them exceptional nutritional reservoirs. While suitable for direct consumption, seaweeds are often processed to develop various products for the food, pharmaceutical, nutraceutical, and hydrocolloid industries. However, their complex structure and composition can hinder nutrient bioavailability. Mild processing or extraction methods could im-

prove their nutritional value. Typically, members of the Phaeophyceae group form a significant portion of the global seaweed population, especially in tropical regions (Sethi, 2012). Despite their benefits and potential, specialized protocols are needed to isolate specific components from seaweed biomass (Matos et al., 2021).

The demand for seaweeds has recently surged, driven by their use in the pharmaceutical and agri-food sectors. This increase is due to their role in functional foods and their antioxidant properties. Seaweed extracts exhibit various pharmacologic effects, such as anti-bacterial, anti-cancer, anti-fungal, anti-viral, anti-inflammatory, anti-coagulant, hypoglycaemic, antioxidant, hypolipidemic, anti-melanogenic, neuroprotective, and hepatoprotective activities (Chakraborty & Paulraj, 2010; Liu, Heinrich, Myers, & Dworjanyn, 2012; Chakraborty, Praveen, Vijayan, & Rao, 2013). Additionally, seaweeds provide dietary iodine and fibre, enhancing food quality (Gupta & Abu-Ghannam, 2011) and also rich in essential nutrients, including calcium, potassium, iron, soluble fibers, polyunsaturated fatty acids, and easily digestible proteins (Zubia, Payri, Deslandes, & Guezennec, 2003). The cellular structures of seaweeds are composed of complex biomolecules, including sulphated polysaccharides, branched moieties, proteins, and bound ions, making bioactive compound extraction challenging (Rodrigues et al., 2015). Aqueous extraction is cost-effective, environmentally friendly, and food-compatible but often less effective in extraction and selectivity (Matos et al., 2021). Organic solvent extraction, using solvents like diethyl acetonitrile, ether, and benzene, offers higher yield and selectivity but is unsuitable for food applications and poses environmental risks (Wijesinghe & Jeon, 2012; Rodrigues et al., 2015).

Developing cost-effective and environmentally sustainable extraction methods is crucial for ensuring food safety, minimizing energy consumption, meeting public health standards, and preserving the environment. Aqueous enzyme-assisted extraction has emerged as a viable green approach, eliminating the need for harsh solvents, acids, or bases (Nguyen et al., 2021; Steinbruch et al., 2023). This method uses tailored combinations of cellulase, protease, and pectinase enzymes to hydrolyze the cell wall, disrupting cells and releasing cellular constituents (Nguyen et al., 2021). Operating at mild pressures and temperatures, this technique reduces energy usage and prevents the degradation of extracted compounds (Nguyen et al., 2021). Moreover, it reduces the use of harmful chemicals while producing high-quality products (Nguyen et al., 2020). Food-grade enzymes, such as carbohydrases for cellulose breakdown and proteases for targeting protein structures, have gained significant attention due to their hydrolytic properties (Teixeira-Guedes et al., 2023). This enzymatic approach offers eco-friendly and non-toxic characteristics by eliminating the need for solvents in the extraction process (Getachew, Jacobsen, & Holdt, 2020; Ummat, Sivagnanam, Rajauria, O'Donnell, & Tiwari, 2021; Teixeira-Guedes et al., 2023; Dulanlebit & Hernani, 2023). Enzymes weaken the inflexible and diverse cell wall structures, releasing targeted bioactive compounds and modifying hydrophilic properties to improve solubility without compromising biological integrity (Wijesinghe & Jeon, 2012; Kadam, Tiwari, & O'Donnell, 2013).

This study aimed to evaluate the bioactive potential of a multi-enzyme complex containing two proteases (Flavourzyme® 1000 L & Alcalase® 2.4L FG) and a carbohydrase (Viscozyme® L), individually or in combination, on the brown seaweeds *T. conoides* and *P. tetrastromatica* (Fig. 1). Focusing on these seaweeds from the Mandapam Coast offers a targeted exploration into less-studied varieties by adding geographical relevance and highlighting how environmental factors shape phenolic content and antioxidant activity. This approach may uncover regional variations and enhance understanding of how location influences bioactive compound production in seaweeds, influenced by geographical origin, seasonal changes, species, environmental conditions, and processing methods. Since these seaweeds can be employed as alternatives to synthetic ones for food, pharmaceutical, and cosmetic industries. Their potential health benefits include reducing oxidative stress, preventing chronic diseases, and promoting their use in functional foods and dietary supplements. Utilizing local brown seaweeds supports sustainable harvesting and economic development in coastal communities. The findings guide the development of products with high phenolic content and antioxidant activity, improving shelf-life and nutritional value. This research can also stimulate further biotechnological studies to optimize extraction methods and maximize bioactive compound yields from seaweeds.

Materials and Methods

The brown seaweeds *P. tetrastromatica* and *T. conoides* (Fig. 1) were harvested from the intertidal zone (9°172 N and 79°112 E) along the Mandapam coast, Tamil Nadu, within the Gulf of Mannar, situated on the southeast Indian coast. The seaweeds were carefully cleaned under running water, rinsed using deionized water, and oven-dried at 45°C for 24h (Kumar et al., 2020). The dried seaweeds were powdered in a blender, transferred to a container, and sealed for further analysis.

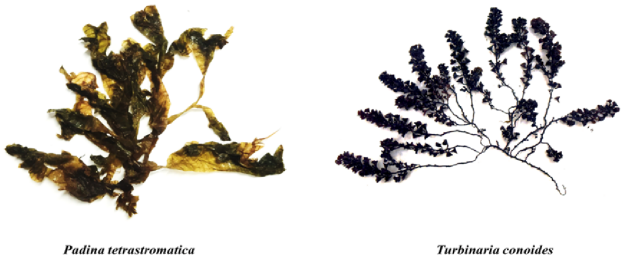

Fig. 1. Brown seaweeds collected from the Mandapam Coast

The extraction process followed the methodology of Habeebullah, Alagarsamy, Arnous, & Jacobsen, (2021) with modifications. Specifically, carbohydrases - viscozyme® L (V) in an acetate buffer at pH 4.5 and 50°C, and proteases - flavourzyme® 1000 L (F) in phosphate buffer at pH 7 and 50°C and alcalase® 2.4 L FG (A) in phosphate buffer at pH 8 and 50°C were used. These enzymes constituted the primary agents investigated in this study. 60mL (1:30 w/v) of the appropriate buffer was used to suspend 2g of the dried seaweed powder. For 10 minutes, the containers were placed in a shaking water bath with a temperature set at 50°C. The corresponding enzyme (100mg) was added and incubated for 20h (5% of seaweed powder). The process ceased by boiling it at 100°C for 10 min and then cooled in an ice bath. After centrifuging (10 min, 3000 rpm), the supernatant was separated, and its pH was set to 7 using HCl/NaOH. All resulting extracts were transferred to separate containers and placed at -80°C. Further, the extracts were freeze-dried for 72h and reconstituted in methanol for further examination. The yield of the extracts obtained was calculated by:

$$\text{Yield (\%)} = \frac{\text{Dried weight of exract}}{\text{Dried weight of seaweed used for extraction}} \times 100$$

The total phenolic content (TPC) was determined by following the method of Singleton & Rossi, (1965). Exactly, 0.2mL of reconstituted seaweed extracts (at a concentration of 5mg/mL) were diluted to 0.5mL, followed by the addition of 2.5mL of Folin's-Ciocalteu reagent (diluted at a 1:10 ratio with water) and 2mL of sodium carbonate (7.5% w/v). The mixture was vortexed and incubated at 50°C for 5 min. Moreover, the absorbance was measured at 760nm using a UV-1601 Shimadzu spectrophotometer. The results were expressed in milligrams of gallic acid equivalents per gram of extracts.

The total antioxidant activity (TAA) was assessed using the phosphomolybdenum technique defined by Prieto, Pineda, & Aguilar, (1999). 0.6M sulfuric acid, 4mM ammonium molybdate, and 28 mM sodium phosphate were mixed and served as the reagent solution. Subsequently, 1 mL of the sample (at a concentration of 5 mg/mL) and an equal amount of reagent solution were mixed and incubated for 90 min at 95°C in a water bath. Incubation resulted in the reduction of molybdenum by forming a distinct blue-green phosphomolybdenum complex. Absorbance was measured at 695nm relative to a control, and the results were reported in milligrams of ascorbic acid equivalent per gram of extract.

The ferric-reducing antioxidant power (FRAP) was assessed as per Oyaizu (1986); seaweed extracts (0.5mL) were combined with 2.5mL of 0.2M phosphate buffer (pH 6.6) and 1% potassium ferric cyanide. This reaction mixture was vortexed for 30 min and incubated at 50°C; then, a 10% trichloroacetic acid (w/v) solution (2.5mL) was added. Subsequently, 2.5mL of the resulting mixture was pipetted and mixed with 2.5mL of distilled water and 0.5mL of 0.1% ferric chloride. Absorbance was measured at 700nm using a UV-1601 Shimadzu spectrophotometer after incubating for 10 min. The results were expressed in milligrams of gallic acid equivalents per gram of extract.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was evaluated by the procedure outlined by Rodeiro et al. (2015). 2mL of 0.2mM methanolic DPPH solution was mixed with 0.5mL of the sample, which was adjusted to 1ml using methanol. The tubes were then kept in the dark for 30 min. Absorbance was measured at 517nm UV-1601 Shimadzu spectrophotometer. The DPPH solution served as the control, while methanol was the blank. The percentage of radical scavenging activity (RSA)

was calculated using the equation mentioned below, and the IC_{50} was represented in mg gallic acid equivalent/g of extracts.

$$\text{RSA (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \times 100$$

The analysis was conducted in triplicates. The data collected were subjected to ANOVA, SPSS version 16.0 (Chicago, IL, USA) statistical software, with a significance level set at $p \leq 0.05$, followed by Duncan's multiple range test.

Results and Discussion

Extracts from the two brown algae were enzymatically obtained using a combination of carbohydrase and two distinct proteases, both independently and in combination. The extraction yield was quantified as the percentage between the dried weight of the extract and the dried weight of the seaweed powder employed for extraction. The extraction yields from various enzyme treatments on *T. conoides* and *P. tetrastromatica* are depicted in Table 1. The maximum output was achieved through the alcalase® extraction method from *T. conoides*, followed by the same from *P. tetrastromatica* with significant differences ($p \leq 0.05$). The lowest yield was significantly similar for *T. conoides* treated with the viscozyme® and *P. tetrastromatica* treated with the enzyme combination. Proteases were reported to show greater efficiency than carbohydrase in solubilizing innate carbohydrates due to the destabilizing effect on the cell wall, which causes the release and solubilization of a higher number of composites (Hardouin et al., 2016). This disparity in efficacy might account for the higher yield in the treatments involving alcalase® compared to the carbohydrase viscozyme®. However, in the case of *T. conoides,* the extraction using enzyme combination (36.0±1.0%) did not surpass the yield obtained from alcalase® (73.3±1.5%). However, it was significantly higher than viscozyme® (24.8±0.5%) and lower than flavourzyme® (46.9±2.2%) enzymatic extracts. In the case of *P. tetrastromatica*, the extraction using enzyme combination (25.5±0.5%) did not significantly exceed the yield obtained from alcalase® (64.2±1.0%), viscozyme® (46.7±1.8%) and flavourzyme® (45.7±1.3%) enzymatic extracts. This could be due to a potential antagonistic effect, the lack of synergy among these enzymes, or the influence of the species employed for this extraction process.

The brown seaweed species *T. conoides*, when treated with alcalase®, exhibited the highest TPC (Table 1), with *P. tetrastromatica* significantly in the second position. *T. conoides* and *P. tetrastromatica* treated individually with viscozyme®, as well as *P. tetrastromatica* treated with the enzyme combination, showed significantly ($p \leq 0.05$) similar TPC, which was the lowest among all other treatments. Both species treated with flavourzyme® displayed significantly similar TPC, while the extracts of *T. conoides* from the enzyme combination and flavourzyme® independently showed no significant variances in TPC. This suggests a potential synergistic or additive effect when these enzymes are combined for phenolic content extraction. However, the enzyme combination yield did not exceed that of the alcalase® treatment alone for both species. According to Gómez-Guzmán, Rodríguez-Nogales,

Table 1. The yield and TPC of enzymatic extracts from brown seaweeds using carbohydrases and proteases.

Treatments	Yield (%)	TPC (mg GAE/g extract)
T. conoides + Viscozyme	24.8±0.5^{a}	1.50±0.9^{a}
T. conoides + Alcalase	73.3±1.5^{e}	3.15±0.2^{e}
T. conoides + Flavourzyme	46.9±2.2^{c}	1.81±0.5$^{b, c}$
T. conoides + Viscozyme + Alcalase + Flavourzyme	36.0±1.0^{b}	1.89±0.2^{c}
P. tetrastromatica + Viscozyme	46.7±1.8^{c}	1.46±0.3^{a}
P. tetrastromatica + Alcalase	64.2±1.0^{d}	2.67±0.2^{d}
P. tetrastromatica + Flavourzyme	45.7±1.3^{c}	1.79±0.7^{b}
P. tetrastromatica + Viscozyme + Alcalase + Flavourzyme	25.5±0.5^{a}	1.53±0.1^{a}

Values are expressed as mean ± SD (n=3). (Superscripts with different alphabets (a – e) indicate significant differences ($p \leq 0.5$) among the enzymatic extracts)

Algieri, & Gálvez, (2018), the phenolic compounds found in algae, such as tannins, phlorotannins, phenolic acids, flavonoids, and catechins, are highly regarded for their health benefits, and these phenolic compounds can differ between various seaweed species. Marine brown algae are particularly identified for high phlorotannin contents composed of the phloroglucinol units (1,3,5-trihydroxy benzene). In the study of Kumar et al. (2020), the TPC varied depending on the extraction solvents, and they ranged from 6.23±0.64 to 32.51±2.05 mg gallic acid equivalent/g of extract. Chandini, Ganesan, & Bhaskar, (2008) also showed similar TPC in brown seaweed extracts, which remained consistent across various solvent systems. Jayarani et al. (2021) state that the enzymatic extracts exhibited a greater TPC (90±2.63 mg gallic acid equivalent (GAE)/g) than supercritical fluid extracts (SFE) and natural hydrophobic deep eutectic solvent (NaDES) extracts and better activity in scavenging free radicals.

Chew, Lim, Omar, & Khoo (2008) noted significant variations in phenol content among different seaweed varieties, indicating that the climate and sunlight directly influence the phenol composites of seaweed. Consequently, the phenol levels in similar seaweed species may vary from one country to another depending on the prevailing climate (Flodin, Helidoniotis, & Whitfield, 1999). Phenolic compounds in seaweeds are thought to vary based on their position along the shoreline. Those growing in the uppermost intertidal areas face intense UV radiation and the risk of drying out, leading to a more excellent production of phenols to cope with these environmental pressures (Connan, Deslandes, & Gall, 2007). Meanwhile, seaweeds in the lower intertidal regions, which stay submerged for more extended periods and experience fewer stressors, require fewer phenols.

Phenolic compounds are considered essential natural antioxidants, and continued research is needed to discover new sources of these substances. Polyphenols have become increasingly common in the human diet and have attracted more interest from consumers and food producers for their wide range of health benefits. These benefits include antioxidant, anti-cancer, anti-bacterial, anti-obesity, anti-allergy, anti-diabetes, anti-inflammatory, anti-HIV, and anti-aging properties (Thomas & Kim, 2011). Three distinct *in vitro* experiments were conducted to obtain the characteristics since the oxidation processes vary.

Antioxidant activity involves generating reductants, which terminate free radical chain reactions (Farvin & Jacobsen, 2015). The antioxidant properties obtained through various enzyme treatments were evaluated based on their capability to reduce Mo^{6+} to Mo^{5+} complexes. This activity was quantified and outlined in Table 2. *P. tetrastromatica* treated with alcalase® significantly exhibited the maximum TAA, followed by *T. conoides* treated with viscozyme®. Conversely, the lowest TAA was significantly similar for extracts of *T. conoides* treated

Table 2. *In vitro* antioxidant properties of enzyme extracts of brown seaweeds obtained from various treatments.

Enzyme treatments	TAA (mg AAE/g extract)	DPPH (mg GAE/g) (IC_{50})	FRAP (mg GAE/g extract)
T. conoides + Viscozyme	6.27±1.1^{d}	4.2±0.7^{c}	2.42±0.8^{b}
T. conoides + Alcalase	3.82±0.3^{a}	3.3±0.7$^{b, c}$	2.11±0.4^{a}
T. conoides + Flavourzyme	3.84±0.1^{a}	1.9±0.7^{a}	2.50±0.5^{b}
T. conoides + Viscozyme + Alcalase + Flavourzyme	4.85±1.2^{c}	4.2±1.0^{c}	4.01±0.2^{d}
P. tetrastromatica + Viscozyme	3.97±0.6$^{a, b}$	1.7±0.3^{a}	3.79±0.9^{c}
P. tetrastromatica + Alcalase	7.34±0.7^{e}	2.5±0.4$^{a, b}$	2.61±0.1^{b}
P. tetrastromatica + Flavourzyme	4.36±0.2^{b}	1.4±0.2^{a}	2.24±0.5^{a}
P. tetrastromatica + Viscozyme + Alcalase + Flavourzyme	4.99±3.3^{c}	1.3±0.5^{a}	3.80±0.3^{c}

The values are presented as mean ± SD (n=3). (Superscripts with different alphabets (a – e) indicate significant differences ($p \leq 0.5$) among the enzymatic extracts)

with alcalase® and flavourzyme® individually and *P. tetrastromatica* treated with viscozyme®. Similarly, *P. tetrastromatica*, when individually treated with viscozyme® and flavourzyme®, demonstrated significantly similar TAA levels ($p \leq 0.05$). Viscozyme®, as a carbohydrase, enhances the release of antioxidants within the extracts by breaking down the cell wall. This underscores the importance of antioxidant activity, which is crucial for preventing free radical chain reactions (Farvin & Jacobsen, 2015), and emphasizes the significance of reducing power as an indicator of a compound's antioxidant potential. The antiradical capacity of various enzymatic extracts studied by Habeebullah et al. (2020) varied between 0.5±0.01 and 12.5±0.6, depending on the species and the enzyme utilized for extraction. Typically, carbohydrases exhibited higher radical scavenging activity compared to proteases, with Neutrase among the proteases demonstrating the greatest radical scavenging activity (Habeebullah et al., 2020).

The capability of a compound to neutralize DPPH radicals relies on its capacity to interact with the unpaired electron of the radical (Park, Shahidi, & Jeon, 2004). A decreased absorbance in the reaction mixture indicates an increased free radical scavenging activity. Table 2 suggested that all three enzymes, individually and in combination, display relatively high DPPH radical scavenging activity. *T. conoides* treated with flavourzyme® and *P. tetrastromatica* treated individually with viscozyme®, alcalase®, flavourzyme®, and in combination revealed the most increased activity, significantly having the least IC_{50} values. Alcalase® treated with both species demonstrated a significant ability to scavenge the DPPH free radical with IC_{50} values of 3.3±0.7 and 2.5±0.4 mg, respectively. Viscozyme®, alcalase®, and the combined enzyme treatment of *T. conoides* exhibited significantly similar IC_{50} values with the lowest activity. Nevertheless, the activity variations among these enzymes are relatively minor, suggesting that each showcases considerable antioxidant potential as per the DPPH assay without substantial differences ($p \leq 0.05$). A prior study found that phlorotannins are phenolic compounds with dual polarity, showing significant antioxidant activity against DPPH radicals (Archana & Vijayalakshmi, 2018).

There was no clear link between the TPC and the radical scavenging activity of the extracts. Some treatments with low TPC showed high radical scavenging activity (Habeebullah et al., 2021). Studies by Lu & Foo (2000) and Siriwardhana, Lee, Jeon, Kim, , & Haw, (2003) found a significant correlation between DPPH scavenging and the TPC of the extracts. Despite *T. conoides* treated with alcalase® having higher phenolic levels in this study, the extracts with lower TPC exhibited markedly greater DPPH scavenging activity. This finding indicates that factors beyond phenolic compounds, such as proteins, peptides, and polysaccharides, could contribute to the DPPH scavenging abilities (Heo, Park, Park, Kim, & Jeon, 2005).

The FRAP assesses antioxidant capabilities by employing the reducing power test to observe the translation of Fe^{3+} to Fe^{2+}. Antioxidant effectiveness is linked to the generation of reductants that aid in halting free radical chain reactions. The ability to reduce is a promising indicator of antioxidant activity. The extracts of *T. conoides* obtained using enzyme combination exhibited the highest FRAP (4.01±0.2 mg GAE/g extract). This outcome could be due to the collaborative impact of proteases and carbohydrases, effectively breaking down cell walls and organelles to release ferric-reducing antioxidants more efficiently. Moreover, *T. conoides* treated with alcalase® and *P. tetrastromatica* treated with flavourzyme® demonstrated significantly lower FRAP. Significantly similar FRAP was exhibited by the extracts of *T. conoides* treated with viscozyme®, flavourzyme®, and *P. tetrastromatica* with alcalase® individually. The individual blend of *P. tetrastromatica* with viscozyme® and the enzyme combination expressed significantly similar FRAP.

Phenolic compounds and carotenoids are well-known for their potent antioxidant capabilities. Naveen, Baskaran, & Baskaran (2021) reported that phenolic extracts from *P. tetrastromatica* had impressive antioxidant effects, even at lower concentrations, which can be a valuable addition to functional foods. They also demonstrated significant DPPH radical scavenging activity, indicating their suitability as a functional food component. Heffernan, Smyth, FitzGerald, Soler Vila, & Brunton (2014) highlighted the potent radical scavenging effects of phenolic extracts from macroalgae.

With increasing health concerns, there has been heightened exploration of new edible sources from both terrestrial and aquatic environments. Despite brown seaweeds being predominantly utilized for industrial purposes, particularly in alginate produc-

tion, their utilization as a food source in India remains limited compared to other South Asian Nations. Limited knowledge about the nutrient content and culinary uses of seaweed in India led to this study, which aimed to examine the bioactive components in the Indian brown seaweeds *T. conoides* and *P. tetrastromatica*. The results are intended to support the practical use in food and pharmaceutical applications.

The extraction of phenolic compounds is influenced by several factors, such as the sample composition, the type and polarity of the extraction solvent, extraction time, and sample size. Extracts were obtained enzymatically using a combination of carbohydrase and two distinct proteases, both individually and together. The highest yield was achieved with the alcalase® enzyme, while the viscozyme® yielded the lowest. Proteases were more effective than carbohydrase in solubilizing native carbohydrates due to the protease's ability to destabilize the cell wall, releasing and solubilizing more compounds. This effectiveness may explain the higher yields observed with alcalase® and flavourzyme® treatments compared to viscozyme®. However, using a combination of enzymes did not exceed the yield from alcalase® alone, though it was higher than using viscozyme® or flavourzyme® individually. This could be due to a potential antagonistic effect or lack of synergy among these enzymes. This study highlights a promising approach for extracting antioxidant-rich extracts using sustainable technologies suitable for various industrial applications. In the food industry, antioxidants can extend shelf life and enhance nutritional value. In the pharmaceutical industry, they can be used to create supplements that reduce oxidative stress linked to various diseases. The nutraceutical industry can design products that promote health and wellness, such as antioxidant-rich supplements for immune support and anti-aging. Enzymatic extraction is generally more sustainable and environmentally friendly, with lower long-term costs due to reduced waste and energy requirements. However, chemical extraction may be more cost-effective initially and better suited for large-scale industrial applications. The choice depends on industry needs, production scale, and environmental regulations. Enzyme-assisted extraction offers significant environmental benefits by reducing waste, lowering energy consumption, and promoting sustainable resource utilization. These advantages support the transition to eco-friendly practices across various industries, contributing to a more sustainable and resilient future.

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