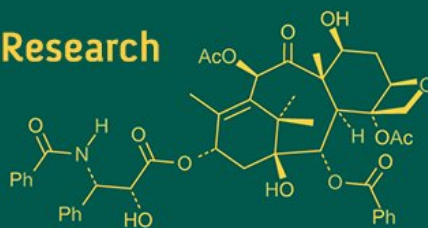


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Characterization of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* as functional ingredients for companion animal diets

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Abstract

Growing environmental constraints such as climate change, diminishing arable land, and increasing competition among food, feed, and biofuel sectors have intensified the demand for sustainable and functional alternative feed resources. Marine macroalgae offer a promising solution due to their rich nutritional profile, diverse bioactive compounds, and environmentally sustainable production system. The present study evaluated the proximate composition, fiber fractions, mineral profile, and antioxidant potential of three tropical seaweeds *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* for their suitability in companion animal nutrition. Seaweed samples were procured from a commercial supplier, thoroughly washed, shade-dried, and ground into fine powder for analysis. Proximate composition was determined using standard AOAC methods, while fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), and insoluble dietary fiber were analyzed using detergent fiber techniques. Mineral profiling was performed using atomic absorption spectroscopy and flame photometry following wet acid digestion. Iodine and cadmium concentrations were also estimated to assess nutritional safety. Antioxidant activity was evaluated through DPPH, ABTS, and FRAP assays along with total phenolic and flavonoid content determination. All analyses were conducted in triplicate, and data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA with significance set at $p < 0.05$. Significant interspecies variation was observed across all parameters. *Gracilaria salicornia* showed the highest crude protein content ($16.8 \pm 0.6\%$) and superior soluble dietary fiber, while *Turbinaria conoides* exhibited higher ash content and mineral richness. *Kappaphycus alvarezii* recorded the highest iodine (980 ± 28 mg/kg) and elevated cadmium levels. Antioxidant activity was significantly higher in *G. salicornia*, followed by *T. conoides*, whereas *K. alvarezii* showed the lowest values. Overall, *G. salicornia* and *T. conoides* demonstrated superior nutritional and functional properties, supporting their potential as functional feed ingredients, while safety evaluation is essential prior to inclusion of *K. alvarezii*.

Keywords: Seaweed; Macroalgae; Companion animal nutrition; Functional feed ingredients; Antioxidant activity

1. Introduction

Climate change, rapid urbanization, shrinking agricultural land, and increasing competition between food, feed, and biofuel sectors are placing unprecedented pressure on conventional animal feed resources. Simultaneously, the growing companion animal population and increasing demand for functional, health-promoting pet foods have intensified the search for sustainable and nutritionally superior alternative feed ingredients. In this context, marine macroalgae (seaweeds) have emerged as promising biomass resources because of their high nutritional value, rich bioactive composition, and environmentally sustainable production systems (Morais *et al.*, 2020; Tanna and Mishra, 2019) [15, 30]. Unlike terrestrial feed crops, seaweeds do not require arable land, freshwater, or intensive fertilizer inputs, making them highly suitable for climate-smart animal nutrition strategies (Wan *et al.*, 2019; Farghali *et al.*, 2022) [8, 32]. Global seaweed production currently exceeds 30 million tonnes annually, largely driven by aquaculture, highlighting their growing importance in future food and feed systems (Corino *et al.*, 2019 [3]; Jönsson and Karlsson, 2023) [13]. India, with an extensive coastline of nearly 8100 km and approximately 700 reported seaweed species, possesses enormous yet underutilized potential for seaweed-based feed applications (Dhingra and Kandiannan,

2021) [6]. However, despite this abundance, seaweeds in India are still predominantly utilized for phycocolloid extraction and agricultural applications, while their direct incorporation into animal nutrition, particularly companion animal feeding systems, remains limited (Pandey *et al.*, 2023) [16]. This underexploited marine biomass therefore offers significant opportunities for developing sustainable, functional, and economically viable pet nutrition systems.

Seaweeds are broadly classified into brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae), each possessing distinct nutritional and biochemical characteristics (Leandro *et al.*, 2020). Among the commercially important tropical seaweeds, *Turbinaria conoides* (brown seaweed), *Gracilaria salicornia* (red seaweed), and *Kappaphycus alvarezii* (red seaweed) have gained increasing attention because of their abundance, adaptability to tropical marine ecosystems, and high nutraceutical potential. Brown seaweeds such as *Turbinaria conoides* are particularly rich in fucoidan, alginate, polyphenols, and antioxidant metabolites that support gut health, oxidative balance, and immune modulation, whereas red seaweeds including *Gracilaria salicornia* and *Kappaphycus alvarezii* are important sources of carrageenans, sulfated polysaccharides, essential minerals, and functional bioactive compounds (Rengasamy *et al.*, 2019; Costa *et al.*, 2021) [4, 21]. In general, seaweeds contain appreciable quantities of proteins, essential amino acids, polyunsaturated fatty acids, vitamins, dietary fiber, and minerals such as iodine, selenium, zinc, iron, calcium, and magnesium, along with diverse bioactive compounds possessing antioxidant, antimicrobial, anti-inflammatory, prebiotic, and immunomodulatory properties (Michalak *et al.*, 2022) [14]. These multifunctional properties make seaweeds attractive as nutraceutical feed ingredients capable of improving digestive health, immunity, metabolic stability, and overall well-being in animals.

The increasing interest in seaweed supplementation is primarily linked to their role as functional feed additives rather than complete feed replacements. Studies in livestock, poultry, aquaculture, and monogastric nutrition have demonstrated that moderate inclusion of seaweed-derived ingredients can improve gut microbiota, antioxidant status, immune responses, growth performance, and product quality (Ribeiro *et al.*, 2021) [22]. Such benefits are particularly relevant in companion animal nutrition, where modern pet owners increasingly demand natural, health-oriented diets that support immunity, gastrointestinal health, healthy aging, and skin and coat quality. Furthermore, seaweeds can partially substitute conventional feed ingredients such as soybean meal and cereal-based components, thereby reducing dependence on terrestrial agricultural resources and easing pressure on food-feed competition (González-Meza *et al.*, 2023) [10]. Beyond their nutritional importance, seaweed cultivation also contributes to carbon sequestration, nutrient recycling, coastal livelihood generation, and marine bioremediation, further strengthening their role in sustainable animal production systems (Sultana *et al.*, 2022; Waqas *et al.*, 2024) [28, 33].

Despite their considerable potential, several challenges limit the broader application of seaweeds in companion animal diets. The nutritional composition of seaweeds varies significantly depending on species, geographical origin, season, environmental conditions, and processing methods, thereby affecting consistency and feed quality (Reddy *et al.*,

2024) [20]. Additionally, some seaweeds may accumulate excessive levels of iodine, arsenic, cadmium, lead, and other potentially toxic compounds, raising concerns regarding safety when incorporated indiscriminately into animal diets (Michalak *et al.*, 2022) [14]. Although processing methods such as washing and thermal treatments can reduce certain contaminants, careful species selection, controlled sourcing, standardized processing, and regulated inclusion levels remain essential to ensure nutritional safety and efficacy (Costa *et al.*, 2021) [4]. Moreover, scientific information regarding the proximate composition, fiber fractions, mineral profile, and antioxidant potential of tropical seaweed species intended for companion animal nutrition remains limited, necessitating detailed species-specific evaluation before dietary incorporation.

Therefore, the present study was undertaken to evaluate the proximate composition, crude fiber fractions, mineral profile, and antioxidant potential of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* for their potential application as functional ingredients in companion animal nutrition.

2. Materials and methods

The study was conducted at the Companion Animal Laboratory, Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. All experimental and laboratory procedures were performed in accordance with the guidelines approved by the IVRI-Institutional Animal Ethics Committee (IAEC) (Approval No. V-137(21)/4/2024-CPCSEA-DADF).

2.1 Collection and processing of seaweed samples

Fresh samples of the seaweeds *Gracilaria salicornia* (red seaweed), *Turbinaria conoides* (brown seaweed), and *Kappaphycus alvarezii* (red seaweed) were procured from AquAgri Processing Private Limited, Delhi, India. The laboratory analyses were carried out at the Companion Animal Laboratory, Division of Animal Nutrition, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. The seaweed samples were taxonomically authenticated prior to analysis. Samples were thoroughly washed with distilled water to remove adhering salts, sand particles, epiphytes, and other impurities, followed by drying in a hot-air oven at 50-60 °C until constant weight was achieved. The dried samples were ground into fine powder using a laboratory grinder and stored in airtight containers for subsequent proximate, fiber, mineral, and antioxidant analyses.

2.2 Proximate composition analysis

Proximate composition of the seaweed samples was determined according to standard AOAC procedures. Parameters analyzed included moisture, crude protein, ether extract, crude fiber, total ash, and nitrogen-free extract. Crude protein content was estimated using the Kjeldahl method, ether extract by Soxhlet extraction, crude fiber through acid-alkali digestion, and total ash by incineration in a muffle furnace at 550 °C. Nitrogen-free extract was calculated by difference.

2.3 Fiber fraction analysis

Fiber fractions including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using the Van Soest detergent fiber method.

The analysis was performed to evaluate the structural carbohydrate composition and dietary fiber characteristics of the seaweed samples.

2.4 Mineral composition analysis

Mineral composition of the seaweed samples was determined following wet acid digestion of the dried seaweed powders using a nitric acid-perchloric acid digestion mixture. Non-volatile minerals including calcium, phosphorus, sodium, potassium, magnesium, iron, zinc, copper, and manganese were quantified using Atomic Absorption Spectrophotometry (AAS), whereas volatile minerals such as selenium and iodine were analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The mineral concentrations were expressed on a dry matter basis.

2.5 Preparation of seaweed extracts for antioxidant analysis

Seaweed extracts were prepared for antioxidant analysis using methanol/ethanol solvent extraction. Briefly, dried seaweed powder was mixed with the extraction solvent and incubated under continuous shaking for 24-48 h at room temperature. The extracts were filtered through Whatman No.1 filter paper and centrifuged, and the supernatants obtained were stored at 4 °C until further antioxidant assays were performed.

2.6 Antioxidant analysis

2.6.1 DPPH radical scavenging activity: Antioxidant activity of the seaweed extracts was evaluated using the DPPH free radical scavenging assay. The reduction in DPPH absorbance was measured spectrophotometrically at 517 nm, and the radical scavenging activity was expressed as percentage inhibition.

2.6.2 Ferric Reducing Antioxidant Power (FRAP): Ferric reducing antioxidant power (FRAP) assay was performed to assess the reducing capacity of the seaweed extracts. Absorbance was recorded at 593 nm, and antioxidant activity was expressed as Trolox equivalent antioxidant capacity.

2.6.3 Total Phenolic Content (TPC): Total phenolic content of the seaweed extracts was estimated using the Folin-Ciocalteu colorimetric method. Absorbance was measured at 765 nm, and the results were expressed as mg gallic acid equivalents per gram of extract.

2.6.4 Total Flavonoid Content (TFC): Total flavonoid content was determined using the aluminum chloride colorimetric assay. Absorbance was recorded at 415 nm, and the flavonoid concentration was expressed as mg quercetin equivalents per gram of extract.

2.7 Statistical analysis

All analyses were carried out in triplicate, and data were expressed as mean $\pm$ standard error. Statistical analysis was performed using one-way analysis of variance (ANOVA) with SPSS software. Differences among mean values were considered statistically significant at $p < 0.05$.

3. Results

3.1 Proximate composition and fiber fraction analysis

The proximate and fiber composition of the three seaweed species revealed significant interspecies variation ($P < 0.05$). *Gracilaria salicornia* exhibited significantly higher crude protein content ($16.8 \pm 0.6\%$) compared with *Turbinaria conoides* ($12.9 \pm 0.4\%$) and *Kappaphycus alvarezii* ($9.8 \pm 0.5\%$). Total ash, crude fiber, NDF, ADF, and insoluble dietary fiber contents were significantly higher in *T. conoides* ($p < 0.05$), indicating its comparatively greater mineral-rich and structural polysaccharide composition. Both *G. salicornia* and *T. conoides* demonstrated superior dietary fiber characteristics compared with *K. alvarezii*, particularly with respect to soluble dietary fiber, where *G. salicornia* recorded the highest value ($21.4 \pm 0.8\%$), followed closely by *T. conoides* ($19.8 \pm 0.7\%$), while *K. alvarezii* showed significantly lower levels ($11.6 \pm 0.5\%$) ($p < 0.05$). Ether extract was significantly greater in *T. conoides*, whereas nitrogen-free extract was highest in *K. alvarezii* ($p < 0.05$). Overall, the findings indicate that *G. salicornia* and *T. conoides* possessed comparatively superior nutritional and functional fiber profiles than *K. alvarezii*, supporting their greater potential as functional ingredients for companion animal nutrition.

Table 1: Proximate composition and dietary fiber fractions of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* (DM basis). Values are expressed as mean $\pm$ standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to determine differences among seaweed species. Statistical significance was considered at $p < 0.05$, while highly significant differences were considered at $p < 0.01$. Different superscripts within the same row indicate significant differences among treatments

Parameter (% DM basis unless stated)	<i>Gracilaria salicornia</i>	<i>Turbinaria conoides</i>	<i>Kappaphycus alvarezii</i>
Dry Matter (%)	89.5 ± 0.8^a	90.2 ± 0.5^a	88.7 ± 0.7^a
Crude Protein (%)	17.4 ± 0.6^a	4.87 ± 0.4^b	9.8 ± 0.5^c
Ether Extract (%)	0.31 ± 0.1^b	0.75 ± 0.1^a	1.2 ± 0.1^c
Total Ash (%)	27.4 ± 1.1^b	35.6 ± 1.3^a	24.6 ± 0.9^c
Crude Fiber (%)	9.0 ± 0.4^b	4.95 ± 0.6^a	3.07 ± 0.3^c
Nitrogen Free Extract (%)	43.4 ± 1.2^b	38.2 ± 1.0^c	56.6 ± 1.5^a
Neutral Detergent Fiber (%)	24.8 ± 0.9^b	31.5 ± 1.2^a	18.9 ± 0.8^c
Acid Detergent Fiber (%)	14.2 ± 0.5^b	18.6 ± 0.7^a	10.5 ± 0.4^c
Soluble Dietary Fiber (%)	22.4 ± 0.8^a	18.9 ± 0.7^b	11.6 ± 0.5^c
Insoluble Dietary Fiber (%)	16.2 ± 0.6^b	22.8 ± 0.9^a	14.4 ± 0.6^c

3.2. Mineral composition analysis

Mineral composition differed significantly among the evaluated seaweed species ($p < 0.05$) (Table 2). *Turbinaria conoides* exhibited significantly higher concentrations of

potassium, sodium, magnesium, iron, zinc, copper, manganese, and selenium compared to *Gracilaria salicornia* and *Kappaphycus alvarezii*. In contrast, *Gracilaria salicornia* recorded significantly greater calcium and

phosphorus contents ($p < 0.05$), indicating superior levels of selected macro minerals. Notably, *Kappaphycus alvarezii* exhibited the highest iodine concentration (980 ± 28 mg/kg), which was significantly greater than both *Gracilaria salicornia* and *Turbinaria conoides* ($p < 0.05$). Additionally, cadmium concentration was significantly elevated in *Kappaphycus alvarezii* (0.41 ± 0.02 mg/kg), whereas the lowest level was observed in *Gracilaria salicornia*. The

findings collectively suggest that while *Turbinaria conoides* possessed superior overall trace mineral richness and *Gracilaria salicornia* demonstrated favorable macro mineral composition, *Kappaphycus alvarezii* showed a greater tendency for iodine and heavy metal accumulation, emphasizing the importance of controlled inclusion levels and safety evaluation prior to dietary application.

Table 2: Mineral composition of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* on dry matter basis. Values are expressed as mean $\pm$ standard error (SE) of triplicate observations. Means within the same row bearing different superscripts (a-c) differ significantly among seaweed species at $p < 0.05$. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test

Mineral	<i>Gracilaria salicornia</i>	<i>Turbinaria conoides</i>	<i>Kappaphycus alvarezii</i>
Calcium (%)	1.92 ± 0.08^a	1.48 ± 0.06^c	1.76 ± 0.07^b
Phosphorus (%)	0.34 ± 0.01^a	0.28 ± 0.01^c	0.30 ± 0.01^b
Potassium (%)	2.15 ± 0.09^b	2.86 ± 0.11^a	1.94 ± 0.08^c
Sodium (%)	1.24 ± 0.05^b	1.68 ± 0.07^a	1.12 ± 0.05^c
Magnesium (%)	0.58 ± 0.02^b	0.82 ± 0.03^a	0.49 ± 0.02^c
Iron (mg/kg)	315 ± 12^b	428 ± 15^a	246 ± 10^c
Zinc (mg/kg)	52 ± 2^b	61 ± 3^a	39 ± 2^c
Copper (mg/kg)	8.4 ± 0.3^b	10.2 ± 0.4^a	6.8 ± 0.2^c
Manganese (mg/kg)	21 ± 1^b	29 ± 1^a	17 ± 1^c
Selenium (mg/kg)	0.92 ± 0.04^b	1.14 ± 0.05^a	0.71 ± 0.03^c
Iodine (mg/kg)	460 ± 18^b	712 ± 21^c	980 ± 28^a
Cadmium (mg/kg)	0.18 ± 0.01^c	0.26 ± 0.02^b	0.41 ± 0.02^a

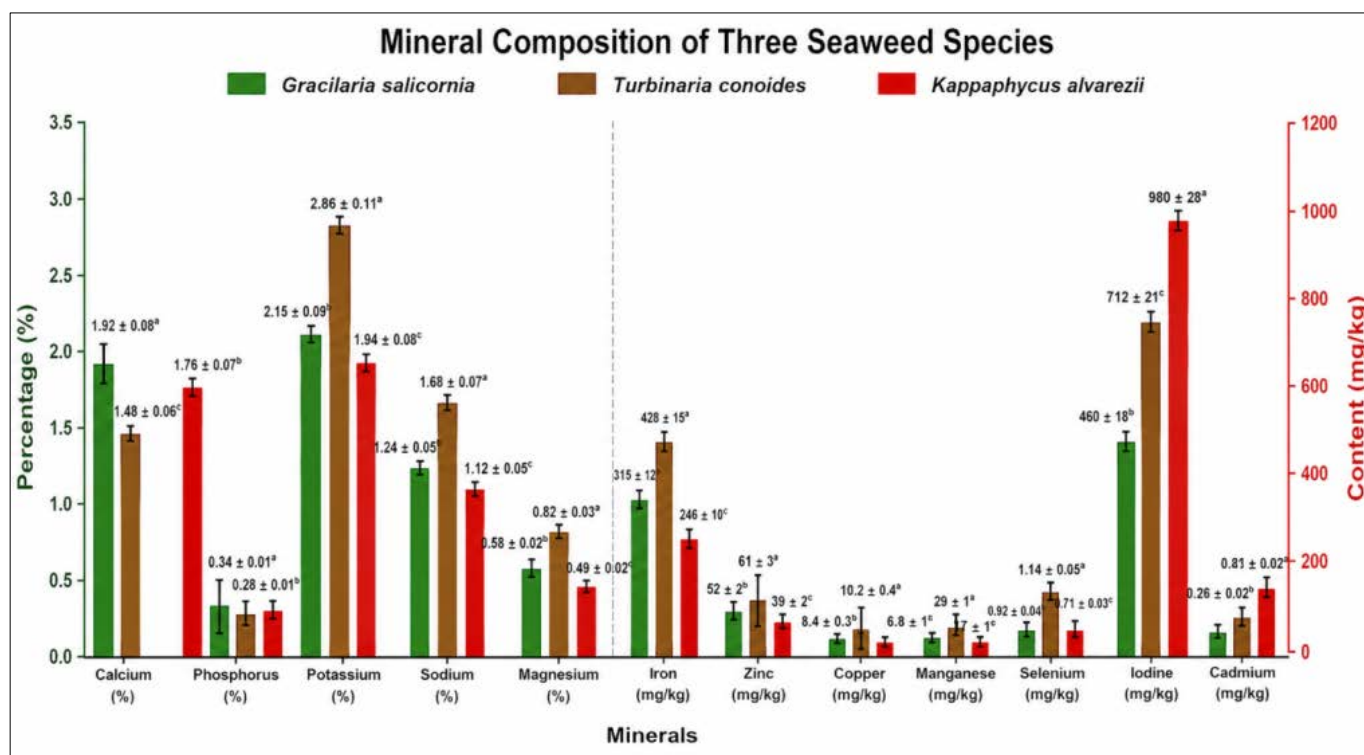


Fig 1: Mineral composition of *Gracilaria salicornia* (GS), *Turbinaria conoides* (TC), and *Kappaphycus alvarezii*

3.3 Antioxidant analysis

Significant differences ($p < 0.05$) were observed among the three seaweed species with respect to antioxidant potential and bioactive compound composition. *Gracilaria salicornia* exhibited significantly higher total phenolic content, DPPH radical scavenging activity, FRAP value, ABTS scavenging activity, and total flavonoid content compared to *Turbinaria conoides* and *Kappaphycus alvarezii*. Although *T. conoides* also demonstrated strong antioxidant activity and substantial phenolic and flavonoid contents, its values remained

significantly lower than those observed in *G. salicornia* ($p < 0.05$). In contrast, *K. alvarezii* recorded significantly lower antioxidant indices and bioactive compound concentrations across all evaluated parameters. Overall, both *G. salicornia* and *T. conoides* demonstrated superior antioxidant and functional bioactive profiles compared with *K. alvarezii*, indicating their greater potential as natural antioxidant-rich functional ingredients for companion animal nutrition.

Table 3: Antioxidant Potential and Bioactive Compound Profile of Selected Seaweed Species Evaluated for Companion Animal Feed Application. Values are presented as mean $\pm$ standard error. Means bearing different superscripts (a-c) within the same row differ significantly ($p < 0.05$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc multiple comparison test

Parameter	<i>Gracilaria salicornia</i>	<i>Turbinaria conoides</i>	<i>Kappaphycus alvarezii</i>
Total Phenolic Content (mg GAE/g extract)	55.4 $\pm$ 1.3 ^a	41.7 $\pm$ 1.2 ^b	15.6 $\pm$ 0.8 ^c
DPPH Radical Scavenging Activity (%)	82.6 $\pm$ 2.7 ^a	76.8 $\pm$ 2.4 ^b	49.5 $\pm$ 1.9 ^c
FRAP Value (μ mol Fe ²⁺ /g)	884.9 $\pm$ 14 ^a	682.3 $\pm$ 13 ^b	214 $\pm$ 9 ^c
ABTS Scavenging Activity (%)	53.8 $\pm$ 2.9 ^a	51.3 $\pm$ 2.6 ^b	53.8 $\pm$ 2.1 ^c
Total Flavonoid Content (mg QE/g extract)	3.64 $\pm$ 0.9 ^a	2.21 $\pm$ 0.8 ^b	1.08 $\pm$ 0.5 ^c

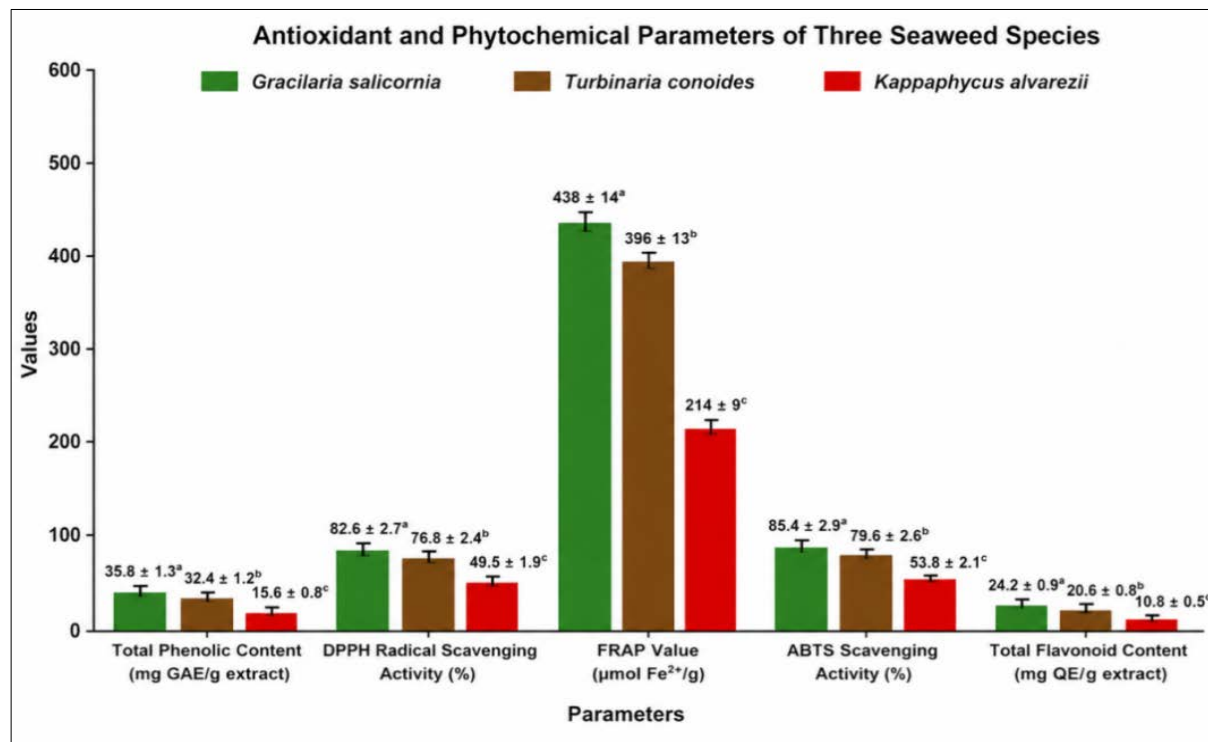


Fig 2: Antioxidant and phytochemical profiles of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* based on total phenolic content, DPPH radical scavenging activity, FRAP value, ABTS scavenging activity, and total flavonoid content

4. Discussion

The growing demand for sustainable and functional feed resources has intensified interest in marine macroalgae as alternative ingredients for animal nutrition. Seaweeds possess several advantages over conventional feed resources because they are rich in bioactive compounds, minerals, dietary fiber, and antioxidant metabolites while requiring neither arable land nor freshwater for cultivation. Their multifunctional nutritional properties have attracted considerable attention in livestock and companion animal nutrition, particularly for the development of health-oriented and climate-smart feeding strategies. However, substantial interspecies variability exists in nutrient composition and bioactive potential, necessitating detailed characterization of individual seaweed species before their incorporation into animal diets. In the present study, marked differences were observed among *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii* with respect to proximate composition, fiber fractions, mineral profile, and antioxidant properties, indicating distinct functional attributes and nutritional applications for each species.

4.1 Proximate composition and fiber fraction analysis

The present study revealed significant species-specific variation in proximate composition and fiber fractions among *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii*, reflecting the influence of

taxonomy, environment, and cultivation conditions. Such variability highlights the need for species-wise evaluation before using seaweeds in companion animal feed formulations. *G. salicornia* showed higher crude protein, consistent with reports describing *Gracilaria* spp. as moderate-to-high protein seaweeds with functional polysaccharides suitable for feed use (Rosemary *et al.*, 2019; Purwaningsih *et al.*, 2024) [18, 23]. In contrast, *K. alvarezii* exhibited higher nitrogen-free extract, indicating carbohydrate dominance typical of carrageenan-rich red algae (Araújo *et al.*, 2022; Islamy *et al.*, 2025) [2, 12]. *Turbinaria conoides* recorded higher ash, crude fiber, NDF, ADF, and insoluble dietary fiber, confirming the mineral-rich and structurally complex nature of brown seaweeds due to high levels of alginate, fucoidan, and related polysaccharides (Diachanty *et al.*, 2017; Rasyid *et al.*, 2025) [7, 19]. In contrast, *G. salicornia* showed superior soluble dietary fiber, followed by *T. conoides*, indicating stronger prebiotic and gut-health potential (Sakthivel and Devi, 2015) [24]. *T. conoides* also exhibited higher ether extract, aligning with reports of diverse lipid fractions and bioactive fatty acids in brown seaweeds (Diachanty *et al.*, 2017) [7]. Overall, *G. salicornia* and *T. conoides* demonstrated superior nutritional and functional profiles compared to *K. alvarezii*. However, reported variability in protein and fiber content of *K. alvarezii* across studies suggests strong dependence on season, strain, and cultivation conditions

(Araújo *et al.*, 2022) ^[2]. This underscores the need for standardized production systems and species-specific evaluation before large-scale application in functional pet nutrition.

4.2. Mineral composition analysis

Significant interspecific variation in mineral composition highlights the importance of species-specific selection of seaweeds for companion animal nutrition. *Turbinaria conoides* exhibited higher concentrations of essential trace minerals including K, Na, Mg, Fe, Zn, Cu, Mn, and Se, consistent with the mineral-rich nature of brown seaweeds (Siahaan *et al.*, 2018; Yoganandham *et al.*, 2018) ^[25, 34]. This supports its potential as a natural source of trace minerals and electrolytes in functional pet feeds, as also reported in *Turbinaria* spp. (Rasyid *et al.*, 2025) ^[19]. In contrast, *Gracilaria salicornia* showed higher Ca and P, in agreement with reports on red seaweeds as rich sources of structural macro-minerals (Aknaf *et al.*, 2024) ^[1]. Its balanced mineral profile, together with favorable fiber and protein content, further supports its role as a multifunctional nutraceutical ingredient. Overall, these findings indicate complementary benefits of brown and red seaweeds for improving dietary mineral balance.

Kappaphycus alvarezii exhibited markedly higher iodine levels, consistent with iodine being a key limiting factor in seaweed-based feeds (Dujardin *et al.*, 2023). While essential for thyroid function, excessive intake may lead to endocrine imbalance if not properly controlled. It also showed higher cadmium levels, aligning with reports of heavy metal bioaccumulation in seaweeds influenced by environmental and processing conditions (Tresnati *et al.*, 2021; Sundhar *et al.*, 2024) ^[29, 31]. Regulatory assessments further emphasize strong species dependence in contaminant accumulation (Guo *et al.*, 2023) ^[11]. Overall, *T. conoides* and *G. salicornia* demonstrated more balanced and desirable mineral profiles for functional feed applications, whereas *K. alvarezii*, despite its nutritional value, requires careful sourcing and controlled inclusion due to elevated iodine and heavy metal content.

4.3 Antioxidant analysis

Marked interspecific variation in TPC, TFC, and antioxidant activities (DPPH, FRAP, ABTS) reflects the species-dependent nature of antioxidant potential in seaweeds and its strong association with phenolic and flavonoid content (González-Meza *et al.*, 2023; Golu *et al.*, 2025) ^[9, 10]. Consistent correlations between TPC/TFC and radical-scavenging activity further confirm phenolics and flavonoids as key contributors to antioxidant capacity (Souid *et al.*, 2024) ^[26]. *Gracilaria salicornia* exhibited the strongest antioxidant activity, supported by earlier reports on *Gracilaria* spp. showing high phenolic and flavonoid content with strong DPPH, ABTS, and FRAP activity (Subramanian *et al.*, 2020) ^[27]. This reinforces its potential as an antioxidant-rich functional ingredient for companion animal nutrition. *Turbinaria conoides* showed moderate antioxidant activity, consistent with brown seaweed studies attributing bioactivity to phlorotannins, fucoxanthin, and other polyphenols (Morais *et al.*, 2020; Paul *et al.*, 2024) ^[15, 17]. These compounds contribute to oxidative stress reduction, immune modulation, and gut health, supporting its functional feed potential (Michalak *et al.*, 2022) ^[14]. In contrast, *Kappaphycus alvarezii* recorded lower TPC, TFC,

and antioxidant activity, consistent with reports showing strong variability due to cultivation conditions, environmental factors, and extraction methods (Das *et al.*, 2023; Reddy *et al.*, 2024) ^[5, 20]. However, improved antioxidant potential under optimized systems indicates strong environmental and processing dependence (Islamy *et al.*, 2025) ^[12]. Overall, *G. salicornia* and *T. conoides* demonstrated superior antioxidant and biofunctional potential compared to *K. alvarezii*, primarily due to higher phenolic and flavonoid content. These findings align with evidence that phenolic-rich seaweeds serve as effective natural antioxidants and sustainable alternatives to synthetic additives in animal feed systems (Morais *et al.*, 2020) ^[15].

5. Conclusion

This study demonstrates clear species-specific variation in the proximate composition, mineral profile, and antioxidant potential of *Gracilaria salicornia*, *Turbinaria conoides*, and *Kappaphycus alvarezii*, highlighting their differential suitability as functional ingredients in companion animal nutrition. *G. salicornia* exhibited higher protein content, favorable soluble dietary fiber, and superior antioxidant activity, indicating strong potential as a nutraceutical-rich functional feed component. *T. conoides* showed enhanced mineral density, structural fiber fractions, and appreciable antioxidant capacity, supporting its role as a valuable natural source of trace minerals and bioactive compounds. In contrast, *K. alvarezii* was characterized by higher carbohydrate, iodine, and cadmium levels, suggesting nutritional potential but also raising concerns regarding safe inclusion without strict quality control. Overall, *G. salicornia* and *T. conoides* emerged as more balanced and functionally promising seaweed candidates for companion animal feed formulations due to their combined nutritional and bioactive advantages. However, the marked variability observed across species underscores the necessity for species-specific evaluation, standardized cultivation practices, and contaminant monitoring prior to large-scale utilization. These findings support the strategic use of selected seaweeds as sustainable, functional alternatives to conventional feed additives in animal nutrition systems.

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