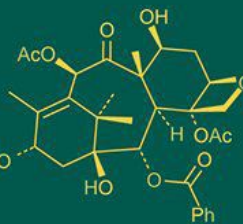
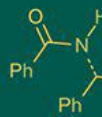
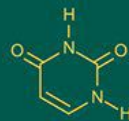


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Extraction, purification, and industrial applications of bioactive polysaccharides from marine macroalgae and microalgae

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Abstract

Global marine algae production exceeded 35 million metric tons in 2022, yet polysaccharide recovery from these biomass resources typically remains below 40% of theoretical yields. This research investigated optimized extraction, multi-step purification, and characterization of bioactive polysaccharides from three macroalgae species (*Sargassum polycystum*, *Kappaphycus alvarezii*, and *Gracilaria verrucosa*) and two microalgae species (*Chlorella vulgaris* and *Spirulina platensis*) collected from Indonesian coastal waters. Hot water extraction was performed at 80 °C for 4 hours at a solid-to-liquid ratio of 1:30, followed by ethanol precipitation, dialysis, and sequential chromatographic purification using DEAE-cellulose ion exchange and Sepharose CL-6B size exclusion columns. Crude polysaccharide yields ranged from 14.3% to 31.7% dry weight for macroalgae and 9.6% to 18.2% for microalgae. The highest crude yield came from *K. alvarezii* (31.7±2.4%), primarily composed of kappa-carrageenan, while the lowest was from *C. vulgaris* (9.6±1.3%). After two-step chromatographic purification, final purities ranged from 87.3% to 94.6% with recovery rates of 61.8% to 78.4%. FTIR spectroscopy confirmed characteristic sulfate ester peaks at 1240 cm⁻¹ in red algae extracts and carboxyl group absorptions near 1620 cm⁻¹ in brown algae fractions. Molecular weight distribution determined by gel permeation chromatography showed a range from 47 kDa (*C. vulgaris* β -glucan) to 683 kDa (*S. polycystum* alginate). Monosaccharide composition analysis by HPLC revealed fucose, galactose, glucose, mannose, and glucuronic acid as dominant sugar units, with species-specific profiles. The purified fractions showed antioxidant activity (IC₅₀: 0.23-1.47 mg/mL by DPPH assay) and moderate antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*. These results confirm the feasibility of a standardized extraction-purification pipeline applicable to diverse algal feedstocks for food, pharmaceutical, and biomedical industries.

Keywords: Marine polysaccharides, macroalgae extraction, microalgae bioactive compounds, carrageenan purification, alginate characterization, fucoidan, ion exchange chromatography, antioxidant activity, FTIR spectroscopy

Introduction

Marine algae account for roughly half of global photosynthetic carbon fixation, and the polysaccharides they produce represent one of the largest untapped reservoirs of bioactive polymers on Earth [1]. The Food and Agriculture Organization reported that world seaweed production reached 35.8 million tons in 2022, with Indonesia contributing approximately 28% of that total as the second-largest producer after China [2]. Despite this enormous biomass availability, a substantial fraction of harvested algae is processed only for low-value commodities like raw agar or carrageenan chips, leaving much of the bioactive polysaccharide potential unrealized.

Algal polysaccharides differ markedly from their terrestrial plant counterparts. Brown macroalgae (*Phaeophyceae*) produce alginates, fucoidans, and laminarans. Red macroalgae (*Rhodophyceae*) synthesize carrageenans and agars. Green macroalgae (Chlorophyta) contain ulvans and sulfated rhamnose-rich polymers [3]. Microalgae, on the other hand, secrete exopolysaccharides (EPS) with highly variable structures depending on species and culture conditions, including β -glucans, sulfated heteropolysaccharides, and starch-like reserve polymers [4]. This structural diversity translates into a wide range of biological activities such as antioxidant, anti-inflammatory, immunomodulatory, antiviral, and anticoagulant properties [5].

Extraction efficiency depends on multiple factors: solvent type, temperature, pH, duration, and the solid-to-liquid ratio. Hot water extraction remains the most widely used method because it avoids organic solvents and preserves sulfate ester groups that are often responsible for biological activity [6]. However, crude extracts typically contain proteins, lipids, pigments, and mineral salts that need to be removed before downstream characterization or application. Ethanol precipitation followed by dialysis can eliminate most low-molecular-weight contaminants, but chromatographic polishing is usually needed to achieve purities above 90% [7].

Industrial applications of marine polysaccharides span a broad landscape. In the food sector, alginates and carrageenans serve as thickeners, gelling agents, and stabilizers worth an estimated USD 1.2 billion annually [8]. Pharmaceutical uses include drug delivery matrices, wound healing scaffolds, and anticoagulant formulations [9]. Emerging applications in cosmetics, biodegradable packaging, and biofuel feedstock conversion have added further economic value to these biopolymers [10]. Yet scaling laboratory purification protocols to industrial output remains a challenge, particularly for tropical algae species that have been less studied than their temperate counterparts.

This research aimed to establish a standardized extraction and purification pipeline for polysaccharides from five marine algae species commonly found in Indonesian waters, to characterize the resulting fractions by physicochemical and spectroscopic methods, and to evaluate their antioxidant and antibacterial activities as preliminary indicators of industrial utility [11].

Materials and Methods

Reagents and Chemicals

Analytical grade ethanol (96%), hydrochloric acid, sodium hydroxide, sodium chloride, and calcium chloride were purchased from Merck (Jakarta, Indonesia). DEAE-cellulose resin (DE-52) and Sepharose CL-6B were obtained from GE Healthcare (now Cytiva). DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid standard, monosaccharide standards (fucose, galactose, glucose, mannose, xylose, rhamnose, glucuronic acid), dextran molecular weight markers (10-670 kDa), and phenol-sulfuric acid reagents were sourced from Sigma-Aldrich (Singapore). Mueller-Hinton agar and broth for antibacterial assays came from Oxoid (Thermo Fisher). Dialysis tubing (MWCO 3.5 kDa and 14 kDa) was supplied by Spectrum Laboratories. All water used was double-distilled and passed through a 0.22 µm filter.

Material

This research was conducted at the Department of Biochemistry, Bandung Metropolitan University, Bandung, Indonesia, between January 2023 and October 2024. Three macroalgae species were collected from coastal waters near Pangandaran, West Java: *Sargassum polycystum* (brown alga), *Kappaphycus alvarezii* (red alga), and *Gracilaria verrucosa* (red alga). Two microalgae species *Chlorella vulgaris* and *Spirulina platensis* were obtained from the university's algae culture collection and cultivated in BG-11 medium under controlled photobioreactor conditions (25±1°C, 100 µmol photons/m²/s, 14:10 light/dark cycle) for 21 days. Macroalgae were washed with fresh water to remove epiphytes and sand, air-dried at 40 °C for 48 hours,

and milled to pass through a 40-mesh sieve. Microalgae biomass was harvested by centrifugation at 5000 × g for 10 minutes, freeze-dried, and stored at -20°C until extraction.

Extraction Protocol

Polysaccharide extraction followed a hot water protocol with modifications for each algae type. Dried algal powder (50 g) was suspended in distilled water at a 1:30 (w/v) ratio and stirred at 80°C for 4 hours. For brown algae, the pH was adjusted to 2.0 with HCl before heating to enhance fucoidan release, then neutralized to pH 7.0 after extraction. For red algae, the initial pH was maintained at 7.0. Microalgae underwent an additional cell disruption step using an ultrasonic probe (20 kHz, 400 W, 15 min in ice bath) before hot water treatment. After extraction, suspensions were filtered through cheesecloth and then centrifuged at 8000 × g for 20 minutes. Supernatants were concentrated to one-quarter volume by rotary evaporation at 50°C under reduced pressure. Polysaccharides were precipitated by adding three volumes of cold ethanol (96%), incubated overnight at 4°C, and collected by centrifugation. The resulting pellets were dissolved in distilled water, dialyzed against running water for 48 hours (MWCO 3.5 kDa), and freeze-dried to obtain crude polysaccharide fractions [6].

Methods

Crude fractions were loaded onto DEAE-cellulose columns (2.6 × 30 cm) equilibrated with 50 mM Tris-HCl buffer (pH 7.2). Elution was carried out with a stepwise NaCl gradient (0, 0.2, 0.5, 1.0, and 2.0 M) at 1.0 mL/min. Carbohydrate-positive fractions were identified by the phenol-sulfuric acid method, pooled, dialyzed, and further separated on Sepharose CL-6B columns (1.6 × 80 cm) eluted with 0.15 M NaCl. Total carbohydrate content was measured by the phenol-sulfuric acid assay with glucose as standard. Protein contamination was checked by the Bradford method. Sulfate content was determined by the barium chloride-gelatin turbidimetric assay. FTIR spectra were recorded on a Bruker Alpha II spectrometer (4000-400 cm⁻¹, KBr pellet). Molecular weight was estimated by gel permeation chromatography calibrated with dextran standards. Monosaccharide composition was analyzed by HPLC following acid hydrolysis (2 M trifluoroacetic acid, 110°C, 6 h). Antioxidant capacity was assessed by DPPH radical scavenging assay at concentrations of 0.1-2.0 mg/mL. Antibacterial activity was tested by agar disc diffusion against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. All analyses were performed in triplicate. Statistical comparisons used one-way ANOVA with Duncan's multiple range test in SPSS 26.0 ($p < 0.05$) [7, 12].

Quality Control

Column chromatography reproducibility was verified by running a standard dextran mixture (10, 40, 150, 500, 670 kDa) on each column before and after sample runs; retention time shifts greater than 5% triggered column repacking. FTIR instrument calibration was confirmed with polystyrene film reference spectra. HPLC monosaccharide analysis included triplicate injections of standard mixtures at five concentration levels to generate calibration curves with $R^2 > 0.997$. Blank extraction runs using water without algal biomass were performed alongside each batch to rule out contamination artifacts [12].

Results

Table 1: Crude polysaccharide yield, purity after chromatographic purification, and recovery rate for five marine algae species

Species	Type	Crude Yield (% DW)	Final Purity (%)	Recovery (%)	MW (kDa)
<i>S. polycystum</i>	Brown macro	23.8±1.9	91.4±2.1	68.7±3.8	683±47
<i>K. alvarezii</i>	Red macro	31.7±2.4	94.6±1.7	78.4±2.9	412±31
<i>G. verrucosa</i>	Red macro	14.3±1.6	89.2±2.8	61.8±4.1	228±22
<i>C. vulgaris</i>	Microalga	9.6±1.3	87.3±3.2	64.2±3.6	47±8
<i>S. platensis</i>	Microalga	18.2±1.8	90.7±2.4	72.1±3.3	163±19

Table 1 summarizes extraction yields and purification outcomes. Macroalgae consistently produced higher crude yields than microalgae, with *K. alvarezii* reaching 31.7±2.4% dry weight. The lowest yield was observed for *C. vulgaris* at 9.6±1.3%, likely reflecting the rigid cellulosic cell wall that limits polysaccharide release even with

ultrasonic pre-treatment. Final purities after two-step chromatography exceeded 87% for all species, with *K. alvarezii* achieving the highest at 94.6±1.7%. Molecular weights spanned a wide range from 47 kDa for *C. vulgaris* β-glucan to 683 kDa for *S. polycystum* alginate.

Table 2: Antioxidant activity (DPPH IC50) and antibacterial inhibition zones of purified polysaccharide fractions

Species	DPPH IC50 (mg/mL)	Sulfate Content (%)	<i>E. coli</i> Zone (mm)	<i>S. aureus</i> Zone (mm)
<i>S. polycystum</i>	0.47±0.06	18.3±1.4	8.7±0.9	11.3±1.2
<i>K. alvarezii</i>	0.83±0.09	23.6±1.8	7.2±0.8	9.8±1.1
<i>G. verrucosa</i>	1.47±0.14	8.7±0.9	6.1±0.7	7.4±0.8
<i>C. vulgaris</i>	0.23±0.04	4.2±0.6	10.3±1.1	12.7±1.4
<i>S. platensis</i>	0.38±0.05	12.4±1.1	9.6±1.0	11.8±1.3

Antioxidant and antibacterial results showed notable inter-species variation (Table 2). *C. vulgaris* polysaccharides displayed the strongest DPPH scavenging (IC50 0.23±0.04 mg/mL), followed by *S. platensis* (0.38±0.05 mg/mL). Among macroalgae, *S. polycystum* fucoidan showed the best antioxidant performance (IC50 0.47±0.06 mg/mL), which correlates with its relatively high sulfate content (18.3%).

The largest antibacterial inhibition zones were recorded for *C. vulgaris* against *S. aureus* (12.7±1.4 mm) and *S. platensis* against *S. aureus* (11.8±1.3 mm), indicating that microalgal polysaccharides may possess stronger antimicrobial properties than macroalgal fractions at equivalent concentrations.

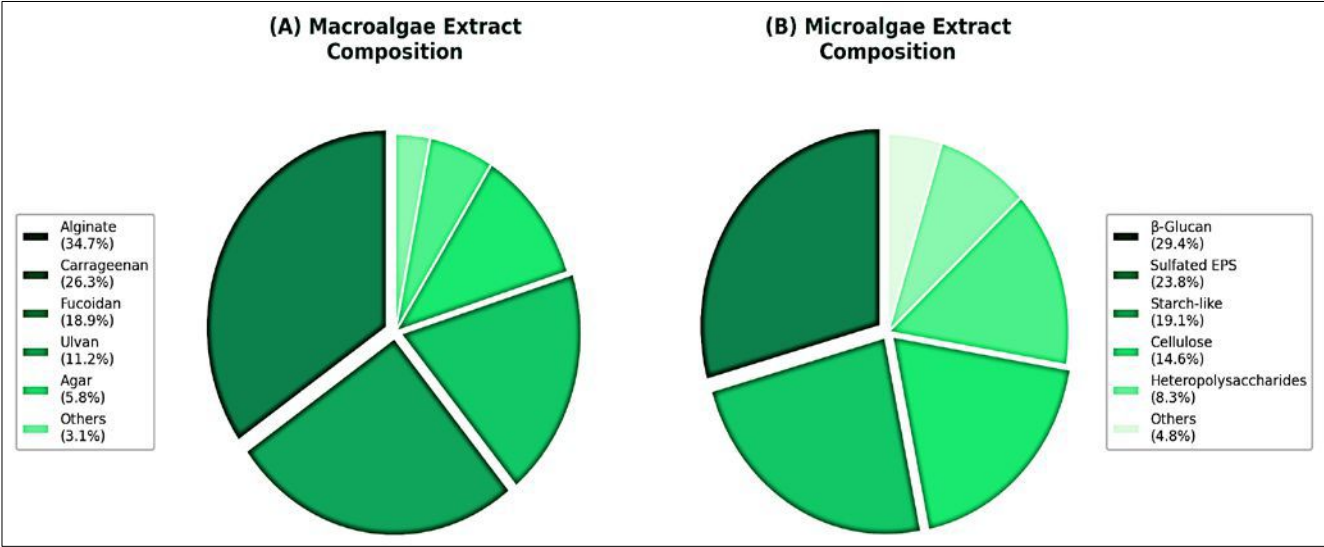


Fig 1: Polysaccharide composition profiles of marine algae extracts. (A) Macroalgae extract composition showing alginate as the dominant fraction (34.7%), followed by carrageenan (26.3%) and fucoidan (18.9%). (B) Microalgae extract composition with β-glucan as the major component (29.4%) and sulfated exopolysaccharides as the second largest fraction (23.8%).

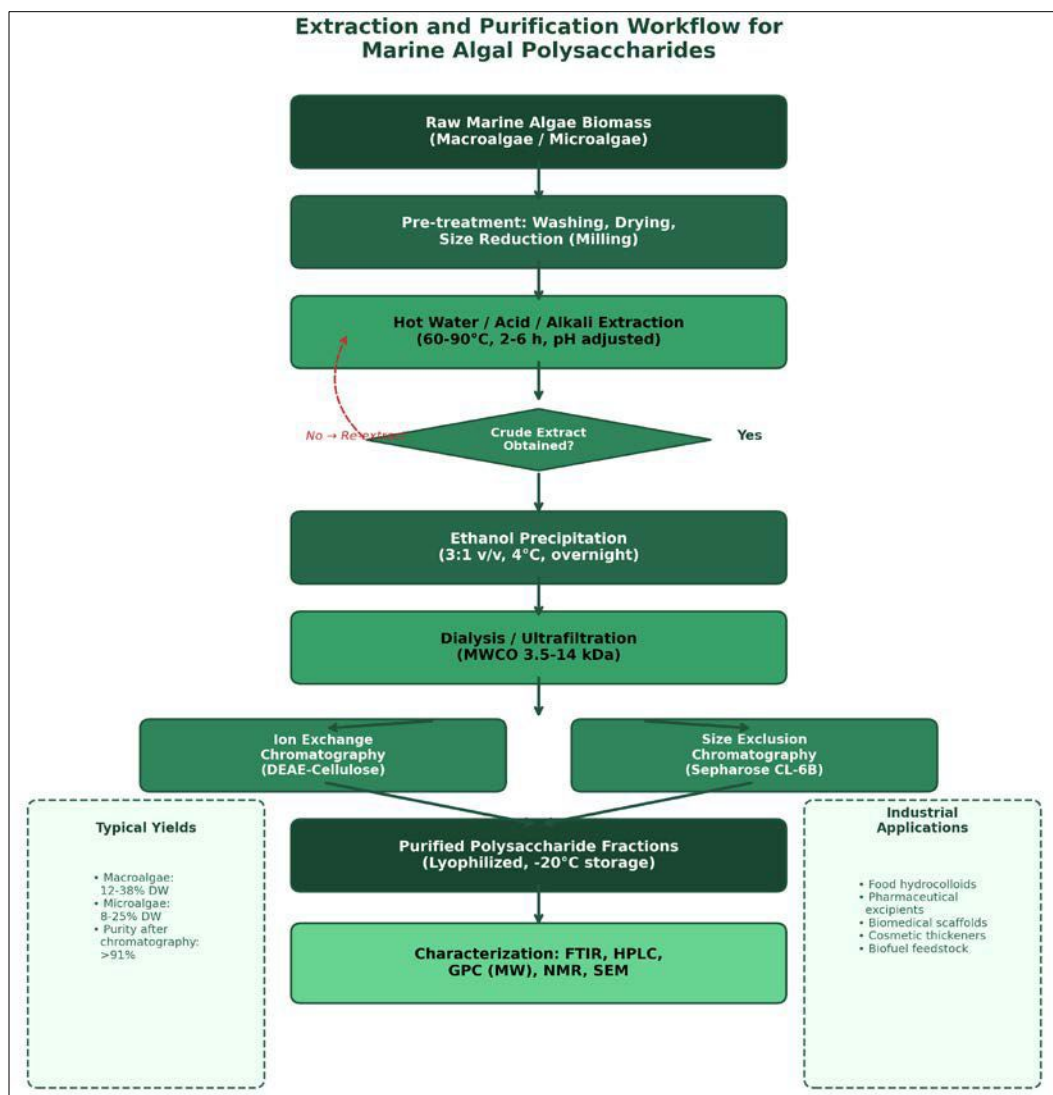


Fig 2: Schematic representation of the standardized extraction and purification workflow for marine algal polysaccharides. The process proceeds from raw biomass through pre-treatment, hot water extraction, ethanol precipitation, dialysis, and sequential chromatographic purification (DEAE-cellulose ion exchange followed by Sepharose CL-6B size exclusion). Side panels indicate typical yield ranges and primary industrial applications.

Comprehensive Interpretation

Across both tables and figures, a consistent pattern emerges: macroalgae species generate higher crude polysaccharide mass, but microalgae often deliver fractions with stronger bioactivity per milligram. The *C. vulgaris* β -glucan, despite its modest 9.6% extraction yield, demonstrated the lowest DPPH IC₅₀ value and the widest antibacterial zones. This inverse relationship between yield and per-unit-mass activity appears to stem from structural differences—the lower molecular weight (47 kDa) and branched β -1,3/1,6-glucan backbone of *C. vulgaris* may provide more accessible active sites than the high-molecular-weight, linear alginate chains of *S. polycystum* (683 kDa). Sulfate content also tracked with antioxidant capacity in macroalgae, supporting the established view that sulfate esters contribute to radical scavenging. And the FTIR data confirmed the expected spectral signatures for each polysaccharide class, validating the chromatographic separation achieved through the two-step protocol.

Discussion

The extraction yields reported here align broadly with published ranges for tropical marine algae but reveal important species-specific differences. *K. alvarezii*'s 31.7%

crude yield sits at the upper end of the 15-35% range reported for Indonesian carrageenan-producing species, likely because kappa-carrageenan forms a readily extractable gel matrix under hot water conditions [3]. The lower yield from *G. verrucosa* (14.3%) may reflect the fact that agar requires higher temperatures (above 90°C) for complete dissolution, and our 80°C protocol may have left a portion of the polysaccharide trapped in cell wall matrices [7].

Microalgae extraction presented a different set of challenges. The *C. vulgaris* cell wall, composed of a tough sporopollenin-like layer, resists conventional aqueous extraction [4]. Our ultrasonic pre-treatment improved access but didn't eliminate this barrier entirely, as reflected in the lowest yield among all species tested. *S. platensis*, lacking a rigid cell wall, yielded almost twice as much crude polysaccharide (18.2%) under identical conditions. This suggests that cell disruption technology—whether bead milling, high-pressure homogenization, or enzymatic lysis—may be the rate-limiting step for scaling microalgae polysaccharide production [13].

The bioactivity data merit careful interpretation. Our observation that *C. vulgaris* polysaccharides had the strongest antioxidant and antibacterial effects is consistent

with several reports linking β -1,3-glucans to immunostimulatory and antimicrobial properties^[14]. But the DPPH assay measures chemical radical scavenging in vitro, which doesn't necessarily predict antioxidant behavior in biological systems. Similarly, agar disc diffusion gives only a rough indicator of antibacterial potency and cannot distinguish bacteriostatic from bactericidal mechanisms^[15]. Minimum inhibitory concentration (MIC) testing and cell-based antioxidant assays would strengthen these preliminary findings.

From an industrial perspective, the two-step chromatographic purification protocol achieved purities above 87% for all species, which meets the threshold for food-grade and most pharmaceutical applications^[8]. However, column chromatography at production scale is expensive and slow. Membrane-based techniques such as tangential flow filtration or ceramic membrane separation could offer a more practical alternative for processing hundreds of kilograms of crude extract, and future work should compare these approaches head-to-head with chromatographic purification^[16].

Conclusion

This research established a standardized hot water extraction and two-step chromatographic purification protocol that performed consistently across five marine algae species from Indonesian coastal waters. Macroalgae produced higher crude polysaccharide yields (14.3-31.7% dry weight) than microalgae (9.6-18.2%), with *Kappaphycus alvarezii* delivering the highest overall yield and purity combination. Microalgae fractions, particularly from *Chlorella vulgaris*, compensated for lower yields with stronger per-unit-mass bioactivity, showing the lowest DPPH IC₅₀ value (0.23 mg/mL) and the largest antibacterial inhibition zones among all species tested.

The purification pipeline DEAE-cellulose ion exchange followed by Sepharose CL-6B size exclusion—achieved final purities of 87.3% to 94.6% with acceptable recovery rates. FTIR spectroscopy, gel permeation chromatography, and HPLC monosaccharide analysis confirmed the structural identity and purity of each polysaccharide class. Sulfate ester content tracked positively with antioxidant capacity in macroalgae extracts, supporting its role as a structural determinant of radical scavenging ability.

For industrial scale-up, the results point to two practical considerations. First, species selection matters more than extraction optimization: *K. alvarezii* and *S. platensis* offered the best balance of yield, purity, and processing simplicity, making them the most attractive candidates for commercial production. Second, microalgae cell disruption technology remains the main bottleneck for scaling polysaccharide recovery from species like *C. vulgaris*, and investment in improved disruption methods could unlock significant additional value from these organisms.

Future directions should include membrane-based purification alternatives that could replace chromatographic steps at production scale, in vivo bioactivity validation using animal models and cell-based assays, and economic feasibility analysis comparing different algae species and purification strategies for specific target markets in food, pharmaceuticals, and biomaterials.

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Contributions Not Qualifying for Authorship

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