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Enzyme-assisted extraction of polyphenols from *Sargassum mcclurei*: antioxidant and alginate lyase inhibition activities

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ABSTRACT

Sargassum mcclurei, a brown seaweed, is a rich source of bioactive polyphenols with promising applications in pharmaceuticals and functional foods. This study used enzyme-assisted extraction (EAE) method to enhance yield and bioactivity, followed by ethyl acetate fractionation. The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method, and antioxidant activity was evaluated through total antioxidant capacity, DPPH radical scavenging, and Fe^{2+} chelation assays. The inhibitory effect on alginate lyase activity was assessed by measuring residual enzyme activity after incubation with polyphenol extracts. The ethanol extraction yielded 2.66% (w/w dry algae), while the ethyl acetate fraction accounted for 37% (w/w ethanol extract). The ethyl acetate fraction exhibited a higher TPC (499.61 ± 1.45 mg PGE/g extract) compared to the ethanol extract (303.04 ± 1.58 mg PGE/g extract) and demonstrated superior antioxidant activity (203.24 ± 1.47 mg AAE/g extract, IC50 = 30.38 µg/mL). Additionally, alginate lyase activity was inhibited dose-dependently, with a 40% reduction observed at 0.5 mg/mL of polyphenol extract. These results highlight the effectiveness of EAE in improving polyphenol yield and bioactivity. The strong antioxidant and enzyme inhibitory properties of *S. mcclurei* polyphenols suggest potential applications in oxidative stress management, microbial biofilm inhibition, and the development of natural therapeutics.

Keywords: Alginate lyase inhibition, antioxidant activity, enzyme-assisted extraction, polyphenols, *Sargassum mcclurei*.

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INTRODUCTION

Marine algae are increasingly regarded as valuable sources of bioactive compounds with potential applications across various fields, including pharmaceuticals, nutraceuticals, and environmental technologies [1]. Among the diverse species of macroalgae, *Sargassum mcclurei*, a brown seaweed found in tropical marine environments, has emerged as a promising candidate for exploring health-related benefits [2, 3]. Compounds derived from brown algae, particularly polyphenols such as phlorotannins, exhibit a wide range of biological activities, including strong antioxidant capacity and the ability to influence enzymatic functions [4].

The extraction of polyphenolic compounds has traditionally relied on organic solvents, a practice that raises concerns about environmental impact and extraction efficiency [5]. Recent advancements in green chemistry have highlighted enzyme-assisted extraction (EAE) as an innovative and sustainable approach. By employing specific enzymes to disrupt cellular matrices, EAE enhances the release of bioactive compounds while preserving their functional integrity [6–8]. This method aligns with modern priorities for sustainable and eco-friendly extraction technologies.

The present study investigates the application of enzyme-assisted ethanol extraction to obtain polyphenols from *S. mcclurei*. The bioactivity of the extracted polyphenols was evaluated through their antioxidant properties and inhibitory effects on alginate lyase activity. Antioxidants are recognized for their critical role in countering oxidative stress, a major factor in chronic diseases [9]. At the same time, alginate lyase inhibitors potentially disrupt biofilm formation by pathogenic microorganisms [10]. The dual focus of this research - optimization of extraction techniques and exploration of multifunctional bioactivities - addresses current gaps in marine bioproduct development.

By exploring these aspects, the study contributes to the sustainable utilization of marine resources and provides insights into developing natural compounds for applications in health and industry. The findings from this research highlight the potential of *S. mcclurei* as a source of bioactive compounds and support its inclusion in broader biotechnological and pharmaceutical efforts.

MATERIALS AND METHODS

Material

Brown alga *Sargassum mcclurei* Setchell 1933 was harvested from the coastal area in Khanh Hoa province in May 2022 and identified by Dr. Vo Thanh Trung (Institute of Oceanography, Vietnam). The whole alga was washed with water to remove impurities, air-dried in the shade. Then the sample was ground to powder and kept in a refrigerator at 4^{o}C until further use.

General experimental procedures

All experiments were conducted using standardized laboratory protocols to ensure accuracy and reproducibility. The major equipment used in this study included a temperature-controlled shaker (N-Biotek, Korea) for enzymatic extraction, a UV-Vis spectrophotometer (Jenway, UK) for absorbance measurements, and centrifugation (Hermle, Germany) was carried out using high-speed centrifuge to separate extracts. All chemicals and reagents used in the study were of analytical grade, and enzymatic reactions were conducted under controlled pH and temperature conditions to optimize polyphenol extraction and bioactivity assessments.

Methods

Enzyme assisted - ethanol extraction of polyphenol

Twenty grams of dried alga were suspended in 100 mL of the phosphate-citrate buffer pH 6. An enzyme mixture consisting of 5% (v/w) cellulase Cellic®CTec2 (Novozyme, Denmark) and 0.35% alginate lyase (recombinant enzyme) was added and

incubated for 24 h on a temperature-controlled horizontal mixer. The reaction was then heated at 90°C for 10 min to precipitate the protein and centrifuged. The supernatant was collected separately for polysaccharide evaluation. The algal residue was treated with ethanol 80% (200 mL) for 24 h. The ethanol extract (0.53 g) was collected, evaporated, and freeze - dried. Ethanol extract (0.4 g) was solubilized in ethanol and further fractionated by ethyl acetate with a ratio of 1:1 (v/v). Ethyl acetate fraction (148 mg) was evaporated and stored at 4°C until analyzed.

Total phenolic content

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu colorimetric assay, as described by Prieto et al., (1999) [11], with minor modifications. Briefly, 200 µL of the extract solution (1 mg/mL in 80% ethanol) or standard solution (phloroglucinol, 0.01–0.10 mg/mL) was mixed with 1 mL of diluted Folin-Ciocalteu reagent (1:10 in deionized water) in a 1 mL glass cuvet. The reaction mixture was incubated at room temperature in the dark for 5 minutes. After incubation, 800 µL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was further incubated at room temperature for 30 minutes in the dark. The absorbance of the final reaction mixture was measured at 765 nm using a UV-Vis spectrophotometer with a 1 cm path length. The TPC was calculated using a standard curve prepared from phloroglucinol and expressed as milligrams of phloroglucinol equivalents (mg PGE) per gram of extract or seaweed.

Antioxidant activities

Total antioxidant capacity: The total antioxidant capacity of the extracts was measured using the phosphomolybdenum complex method, as described by Prieto et al., (1999) [12]. In this assay, 100 µL of the extract at various concentrations was mixed with 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the standard, and results were expressed as mg ascorbic acid equivalents (mg AAE) per gram of extract or seaweed.

DPPH radical scavenging activity: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined following the method of Yen and Chen (1995) [13]. To determine the DPPH radical scavenging activity, 1.0 mL of the sample was mixed with 3.0 mL of 25 mg/L DPPH in methanol. The mixture was kept in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated using the following formula. The DPPH radical scavenging activity was calculated using the following formula:

$$\%DPPH\ scavenging\ activity = \left(1 - \frac{A\,sample - A\,blank}{A\,control}\right) \times 100$$

where: A control: absorbance of the *DPPH* solution without the sample; *A sample*: absorbance of the sample with *DPPH*; *A blank*: absorbance of the sample without *DPPH*.

The inhibition curves were used to calculate the IC50 value, which is defined as the concentration required to scavenge 50% of DPPH radicals.

Ferric ion (Fe^{2+}) chelation activity: The Fe^{2+} chelation activity was evaluated according to the method of Zhu et al., (2002) [11]: Briefly, 1 mL of phosphate buffer (pH 7.2) and 1 mL of 1% (w/v) potassium ferricyanide were added to 1 mL of the sample solution. The mixture was then incubated at 50°C in a water bath for 20 minutes. After incubation, 1 mL of 10% trichloroacetic acid was added, followed by mixing with 0.6 mL of distilled water and 0.16 mL of ferric chloride. The absorbance of

the final solution was measured at 655 nm, and $FeSO_4$ was used as standard.

Inhibitory effect on alginate lyase activity

The enzyme was incubated with the extracts at diferent concentration (0–0.5 mg/mL) for 20 min to investigate the inhibitory effects of polyphenol extract on alginate lyase activity. Then, 0.025 mg/mL of the enzyme was mixed with 2 mg/mL of alginate (Sigma) in 20 mM UB4 buffer. The enzyme activity was determined by measuring the absorbance at 235 nm every 30 seconds using a microplate reader for the first 15 minutes. The initial reaction rates, observed as a linear range over 5-minute intervals, were converted to mM per second using an extinction coefficient of 6,150 $M^{-1}.cm^{-1}$ [14].

RESULTS AND DISCUSSION

Brown seaweeds of the genus *Sargassum* are widely recognized as rich sources of bioactive compounds, notably polyphenols, which exhibit potent antioxidant, antimicrobial, and enzyme-inhibitory properties [15, 16]. *Sargassum mcclurei*, a species commonly found along the Vietnamese coastline, including Khanh Hoa province, has gained attention due to its high polyphenol content and potential pharmaceutical applications. Morphologically, *S. mcclurei* is characterized by a brown to yellow-brown thallus, with serrated, lanceolate leaves and small spherical air bladders aiding in buoyancy. The reproductive structures appear in dense clusters, forming reproductive receptacles along the branches [17]. This species thrives in shallow coastal waters and exhibits seasonal growth variations, with the most favorable harvesting period occurring from March to July when biomass production and bioactive compound accumulation reach optimal levels (Fig. 1).

Given its abundance and bioactive potential, *S. mcclurei* is a promising raw material for polyphenol extraction. Enzyme-assisted extraction (EAE) has been employed as an alternative to conventional solvent extraction methods to enhance extraction efficiency and preserve bioactivity. The following sections evaluate the impact of EAE on polyphenol recovery, antioxidant capacity, and alginate lyase inhibitory activity, providing insights into the advantages of enzymatic hydrolysis in marine bioresource utilization.

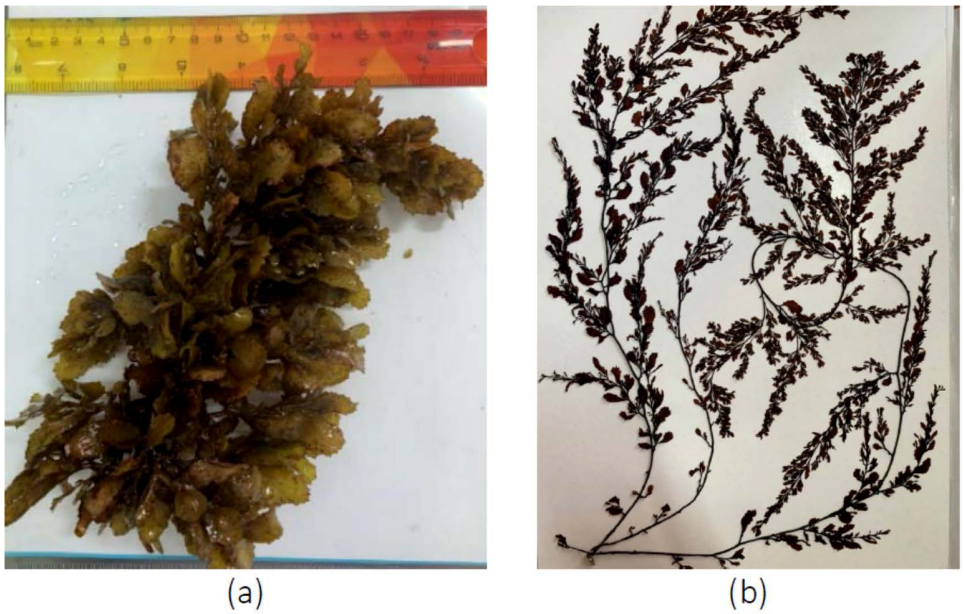

Figure 1. External morphology (a) and specimen (b) of brown algae *S. mcclurei* Setchell 1933 collected at Hon Chong, Nha Trang in May 2022

Polyphenol extraction from *S. mcclurei*

The polyphenol extraction from *S. mcclurei* was carried out using an enzyme-assisted ethanol extraction method followed by fractionation with ethyl acetate. Each extract's extraction yield and total phenolic content of were evaluated (Table 1).

Table 1. Polyphenol extraction yield and total phenolic content from *S. mcclurei*

Sample	Yield (%)	Total phenolic content	
		mgPGE/g extract	mgPGE/g seaweed
Ethanol extract (w/w algae)	2.66	303.04 ± 1.58	8.05 ± 0.04
Ethyl acetate extract (w/w ethanol extract)	37.00	499.61 ± 1.45	3.96 ± 0.07

The extraction of polyphenols from *S. mcclurei* using enzyme-assisted ethanol extraction yielded 2.66% (w/w algae) for the ethanol extract and 37.00% (w/w ethanol extract) for the ethyl acetate fraction. This yield surpasses previously reported values for ethanol extraction of *S. mcclurei* without enzymatic assistance, which was approximately 0.29% (w/w algae) [18]. The enhanced yield in the current study highlights the effectiveness of enzyme-assisted extraction (EAE), particularly cellulase and alginate lyase, breaking down cell walls and releasing bioactive compounds.

Total phenolic content (TPC) is a critical indicator of the antioxidant potential of seaweed extracts. In this study, the TPC of *Sargassum mcclurei* ethanol extract was determined to be 8.05 mg PGE/g seaweed, while the ethyl acetate fraction exhibited 3.96 mg PGE/g seaweed (Table 1). Van et al., (2013) reported a TPC value of 2.34 mg PGE/g dried seaweed for *S. mcclurei* extracted using 90% ethanol [16]. This method suggests that our enzyme-assisted ethanol extraction (EAE) significantly enhances polyphenol recovery, yielding approximately 3.4 times higher TPC than conventional ethanol extraction methods. The increased efficiency can be attributed to the enzymatic breakdown of cell walls, facilitating the release of bound polyphenols. Moreover, the ethyl acetate fraction exhibited a notably higher TPC (499.61 mg PGE/g extract) than the ethanol extract (303.04 mg PGE/g extract), demonstrating the effectiveness of fractionation in concentrating bioactive polyphenols. This disparity suggests that while the ethyl acetate fraction may be more effective at concentrating phenolic compounds, it likely involves a lower extraction yield than the starting seaweed material. Conversely, ethanol extraction provides a more balanced approach, yielding a higher recovery of phenolic compounds in the raw seaweed material. These findings underscore the complementary roles of ethanol and ethyl acetate in optimizing yield and phenolic content concentration, providing valuable insights into selecting solvents for extracting bioactive compounds.

Antioxidant activities

The antioxidant activity of polyphenol extracts (ethanol extract) from *S. mcclurei* was comprehensively evaluated using total antioxidant capacity, DPPH radical scavenging activity, and Fe^{2+} chelation assays. The results demonstrated that the ethyl acetate fraction exhibited superior TAC (203.24 mg AAE/g extract) compared to the ethanol extract (127.17 mg AAE/g extract). This difference suggests that the ethyl acetate fraction contains a higher concentration of bioactive polyphenols per gram of extract. However, when normalized to the dry weight of seaweed, the ethanol extract retained a higher antioxidant capacity (3.25 mg AAE/g seaweed) than the ethyl acetate fraction (1.61 mg AAE/g seaweed), indicating a more significant overall recovery of antioxidants from the algal biomass (Table 2).

The DPPH radical scavenging assay further confirmed these trends. The ethyl acetate fraction showed a significantly lower IC_{50} value (30.38 µg/mL) than the ethanol extract (102.20 µg/mL), indicating stronger radical-scavenging efficiency. This result correlates with the higher

total phenolic content (TPC) of the ethyl acetate fraction (499.61 mg PGE/g extract) compared to the ethanol extract (303.04 mg PGE/g extract). The strong relationship between TPC and antioxidant activity aligns with previous findings that polyphenol-rich fractions typically exhibit enhanced free radical neutralization capabilities.

Table 2. Antioxidant activity of polyphenol extract from *S. mcclurei*

Samples	Total antioxidant capacity		DPPH IC_{50} (µg/mL)	Fe^{2+} equivalent (µg/mL)
	mg AAE/g extract	mg AAE/g seaweed		
Ethanol extract	127.17 ± 0.61	3.25 ± 0.23	102.20	262.68 ± 1.87
Ethyl acetate extract	203.24 ± 1.47	1.61 ± 0.19	30.38	252.68 ± 0.47

Regarding Fe^{2+} chelation activity, the ethanol extract exhibited slightly higher metal chelation ability (262.68 µg/mL) than the ethyl acetate fraction (252.68 µg/mL). This finding contrasts with previous studies where ethyl acetate fractions generally demonstrated superior metal ion chelation due to their concentrated phenolic compounds. The lower chelation activity of the ethyl acetate fraction in this study may be attributed to the selective extraction of phlorotannins while excluding other metal-binding compounds, such as polysaccharides and proteins, which are more soluble in ethanol.

The antioxidant activity of polyphenol extracts from Vietnamese brown seaweeds has been widely documented. Van et al., (2013) reported that *S. polycystum* exhibited the strongest *DPPH* scavenging activity (EC_{50} = 0.08 mg extract/L), while *Dictyota dichotoma* showed the highest total antioxidant capacity (19.6 mg AAE/g dry seaweed [16]. Similarly, *S. microcystum* demonstrated the highest ferric reducing activity (23.1 mg Fe^{2+} equivalent/g seaweed), highlighting its ability to mitigate oxidative stress. In comparison, our study on *S. mcclurei* revealed a strong antioxidant potential, particularly in the ethyl acetate fraction, which exhibited a total antioxidant capacity of 203.24 mg AAE/g extract and a *DPPH* IC_{50} value of 30.38 µg/mL. These values suggest that polyphenols extracted via enzyme-assisted methods retain potent antioxidant properties, reinforcing the effectiveness of enzymatic extraction in enhancing bioactive compound yield and activity. Furthermore, previous studies on Vietnamese *Sargassum* species have reported high antioxidant activity across different extraction methods. For instance, *S. dentifolium* and *S. duplicatum* exhibited high ferric-reducing activity (14.4 mg Fe^{2+}/g and 19.1 mg Fe^{2+}/g, respectively), supporting the role of polyphenols as electron donors in oxidative stress reduction. These findings are consistent with our results, which demonstrate that the ethyl acetate fraction of *S. mcclurei* is highly enriched in antioxidant compounds.

The findings of this study confirm that polyphenol extracts from *S. mcclurei* exhibit strong antioxidant activity, with the ethyl acetate fraction demonstrating superior total antioxidant capacity (TAC), DPPH radical scavenging, and Fe^{2+} chelation compared to the ethanol extract.

Inhibitory effect on alginate lyase activity

The inhibitory effect of the polyphenol extract from *Sargassum mcclurei* on alginate lyase activity was assessed at different extract concentrations (0.1–0.5 mg/mL). As shown in Figure 2, the relative activity of alginate lyase decreased progressively with increasing polyphenol extract concentration, demonstrating a dose-dependent inhibitory effect.

The results show that at the lowest tested concentration (0.1 mg/mL), the alginate lyase retained approximately 90% of its activity, while at 0.2 mg/mL, the enzyme activity dropped to around 75%. A further decrease in activity was observed at 0.3 mg/mL, where the relative activity was reduced to approximately 60%. The inhibition continued with increasing concentrations, reaching around 40% of relative activity at 0.5 mg/mL. These results

suggest that the polyphenol extract effectively inhibits alginate lyase in a concentration-dependent manner.

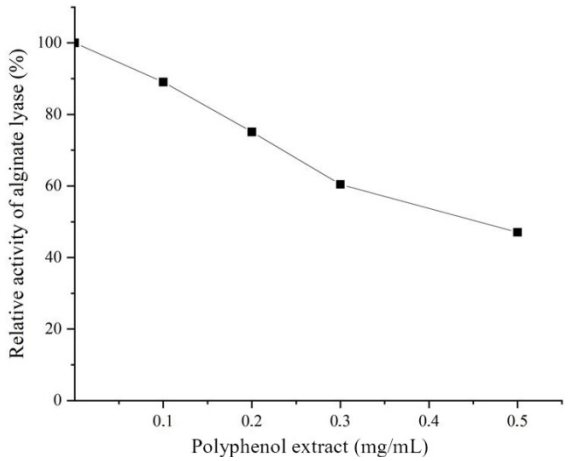

Figure 2. Inhibitory effect of polyphenol extract on alginate lyase activity

This inhibition likely relates to polyphenol-enzyme interactions, mainly through hydrogen bonding and hydrophobic interactions between polyphenols and key active site residues of alginate lyase. Similar inhibitory effects have been reported for other phlorotannin-rich seaweed extracts, where polyphenols interfere with enzymatic activity by forming complexes with proteins, thereby reducing substrate binding affinity and catalytic efficiency [19]. This mode of inhibition has potential implications for antimicrobial and anti-biofilm strategies, as alginate lyase is crucial for bacterial biofilm degradation and pathogenicity [19].

These findings highlight the potential of *S. mcclurei* polyphenols as natural inhibitors of alginate lyase, supporting the further investigation into their biomedical and pharmaceutical applications, particularly in biofilm-related infections and microbial control strategies. Future research should focus on identifying the specific polyphenol compounds responsible for inhibition and elucidating their binding interactions with alginate lyase at the molecular level.

Thus, enzyme-assisted extraction (EAE) in this study significantly enhanced polyphenol yield and bioactivity from *Sargassum mcclurei*, as indicated by the higher total phenolic content (TPC) and antioxidant activity in enzyme-treated extracts. Compared to conventional ethanol extraction, EAE improved extraction efficiency by 3.4 times, consistent with previous findings on marine macroalgae [15]. This improvement is primarily attributed to the enzymatic hydrolysis of structural polysaccharides, particularly cellulose and alginate, which form rigid cell walls in brown seaweeds [7].

Despite these promising results, this study did not comprehensively evaluate key EAE parameters, such as enzyme concentration, enzyme-substrate ratio, and incubation time, which are known to influence polyphenol recovery and bioactivity. Optimizing these factors is essential, as previous research has demonstrated that adjusting enzyme concentration within an optimal range enhances the release of bioactive compounds [8]. However, excessive enzyme loading beyond a threshold can lead to substrate saturation or enzymatic degradation of polyphenols, reducing extraction efficiency [6].

Furthermore, the synergistic effect of multiple enzyme combinations has not been fully explored. While cellulose and alginate lyase effectively break down cell walls, additional enzymatic activities—such as proteases or hemicellulases—may further enhance polyphenol solubility and extraction yield. Multi-enzyme systems have been shown to improve polyphenol recovery by targeting different structural components of the seaweed matrix, warranting further investigation.

To fully harness the potential of EAE, future research should focus on optimizing enzymatic conditions, including enzyme ratios, reaction temperature, and extraction duration, to maximize bioactive compound yield while preserving structural integrity and antioxidant capacity. Additionally, comparative studies on different Sargassum species would provide insights into the scalability and applicability of EAE in marine bioresource utilization. However, extraction methodology remains a crucial determinant of yield. Traditional solvent-based methods often fail to degrade cell walls, leading to lower phenolic recovery. In contrast,

EAE enhances polyphenol yield and preserves bioactivity, supporting its potential as a sustainable and efficient alternative for marine-derived bioactive compound extraction [20].

These findings emphasize the importance of process optimization in enzyme-assisted extraction and highlight its role in advancing sustainable marine bioproduct research.

CONCLUSION

This study demonstrated that enzyme-assisted ethanol extraction (EAE) significantly enhances the yield and bioactivity of polyphenols from *S. mcclurei*. The extraction process yielded 2.66% (w/w dry algae) for the ethanol extract and 37% (w/w ethanol extract) for the ethyl acetate fraction. The ethyl acetate fraction exhibited the highest total phenolic content (499.61 ± 1.45 mg PGE/g extract) and superior antioxidant activity, with a total antioxidant capacity of 203.24 ± 1.47 mg AAE/g extract and a *DPPH* IC_{50} value of 30.38 µg/mL, demonstrating its potent free radical scavenging ability. Furthermore, the polyphenol extract exhibited a dose-dependent inhibitory effect on alginate lyase activity, reducing enzyme activity to 40% at 0.5 mg/mL. This obervation suggests that *S. mcclurei* polyphenols may helpful in microbial biofilm inhibition and disease prevention strategies. Potent antioxidant and enzyme inhibitory properties support their potential use in nutraceutical, pharmaceutical, and biomedical applications. These findings highlight the advantages of EAE over conventional extraction techniques, not only in improving polyphenol recovery but also in preserving bioactivity. Further research should focus on the structural characterization of the active polyphenols, their mechanisms of action, and potential in vivo applications to fully explore their therapeutic potential.

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