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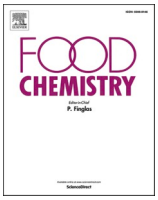

Study of phenolic-polysaccharide interactions in brown seaweed

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ABSTRACT

Brown seaweed has a rich source of bioactives, notably antioxidative phenolic compounds and sulphated polysaccharides. Despite their importance, the interactions between these compounds and their resultant antioxidant activities have not been extensively studied. This research aims to investigate the interaction between seaweed-derived phenolics and polysaccharides in terms of their antioxidant activity, exploring their potential interactions through in silico molecular docking, and validating them using Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS) and differential scanning calorimetry (DSC). Reduced antioxidant activities were observed in the seaweed-derived phenolics and polysaccharides combination. The in silico molecular docking studies and experimental analyses revealed non-covalent interactions, mainly driven by hydrogen bonding between fucoidan and catechin or phloroglucinol. The resulting 'complex' formation varied in size and thermodynamic stability, suggesting structure-dependent interactions between fucoidan and phenolic compounds. This research helps to understand the marine phenolic-polysaccharide interaction, contributing to their effective application in the functional food and nutraceutical industries.

1. Introduction

Seaweed production and utilization have grown tremendously over the past few years, driven by the identification of health-promoting bioactive compounds in seaweed such as phlorotannin, polysaccharides, and fucoxanthins. Brown seaweed (Phaeophyceae), the second largest group of marine algae, encompasses over 2000 species and has been perceived as a form of medicinal food, especially in the Eastern cultures (Rodriguez-Jasso et al., 2011). Growth exposure to harsh marine environmental conditions leads to the accumulation of distinctive phenolic compounds such as phlorotannin that are solely found in brown seaweed (Lee et al., 2023). These compounds have garnered significant attention due to their multitude of bioactivities, such as antioxidant, anti-aging and anti-inflammatory activities (Landa-Cansigno et al., 2023; Subbiah et al., 2024). Additionally, the high salinity of the marine environment has contributed to the development of a unique structural composition and diversity of brown seaweed polysaccharides. For example, fucoidan is a sulphated polysaccharide that helps to entrap water in the cell wall, preventing desiccation (Arata et al., 2017). Beyond its interesting structural features, fucoidan also exhibits multiple biological activities such as antioxidant, antidiabetic, antimicrobial and anti-inflammatory activities (Jun et al., 2018; Obluchinskaya et al., 2022; Xie et al., 2024). Among these diverse biological activities, the antioxidant activity of these compounds is of primary interest. This is because oxidative stress, as caused by excessive production of free radicals in the body is the major contributing factor driving chronic disease development and progression (Forman & Zhang, 2021). Thus, consumption of high antioxidant diet can contribute to reducing oxidative stress and mitigating related diseases (Rudrapal et al., 2022).

During food maceration or food processing which involves high temperature, pressure and/or shearing forces, the disruption of the algae cell wall can lead to interactions between various molecules (Lee et al., 2023). Given the known antioxidant activity of brown seaweed phenolic compounds and polysaccharides, it is important to understand how these molecules might interact and influence their antioxidant activity under these conditions. The research to date has largely focused on the phenolic-polysaccharide interaction in plant systems, while similar interactions in marine sources have not been fully explored. Previous studies examining the combinatorial effect of phenolic compounds and plant-based polysaccharides have reported inconsistent findings with some showing reduced antioxidant activity (Domínguez Avila et al., 2018; Silva et al., 2024) and others reporting the opposite effect (Mercado-Mercado et al., 2020; Mihai et al., 2023). This variability suggests that the synergistic/antagonistic antioxidant effects of phenolic-polysaccharide interactions might be at play and is highly

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dependent on their structural characteristics. Furthermore, it has been found that phenolic compounds mainly interact with polysaccharides via non-covalent interactions such as hydrogen bonding, hydrophobic interactions and the weak *Van der Waals* forces (Zhu, 2018). For example, Fernandes et al. (2020) demonstrated that pectin interacts non-covalently with polyphenols, and similar studies have been conducted in other plant systems such as oat beta glucan, starch, and cellulose (Liu et al., 2018; Ma et al., 2023; Wu et al., 2011). However, marine polysaccharides such as fucoidan are structurally distinct from plant-based polysaccharide, thus, to derive their phenolic interactions from plant polysaccharides would be questionable.

To date, only a few studies have investigated the interactions between marine polysaccharides and phenolic compounds, focusing mainly on fucoidan (Deepika et al., 2019; Gao et al., 2023). However, these studies did not elucidate the detailed mechanism of their interaction or the subsequent effect on antioxidant activity. This highlights a significant research gap that warrants further investigation, particularly in relation to their antioxidant activity. Deciphering this interaction is important for their effective application, especially for functional food and nutraceutical development. This study employed a stepwise approach to investigate seaweed phenolic-polysaccharide interaction, including extraction and characterization of seaweed phenolic compounds and polysaccharides, assessing the antioxidant interaction between them, and elucidating their potential interaction using molecular docking simulation. The latter was then experimentally validated using Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS) and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials and reagents

Brown seaweed samples (*Cystophora siliquosa* and *Phyllospora comosa)* were collected from the Australia coastal region (38°15′54.0″ S 144°40′10.3″ E) during Spring 2023. Organic solvents used for extraction and analysis were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Other chemicals and standards of analytical grade or higher were also sourced from Sigma Aldrich. Fucoidan standard (Fuc_{std}) from *Undaria pinnatifida* was purchased from Sigma Aldrich. Deionized water used was produced by Milli-Q water System (Merck, Milli-Q® Direct).

2.2. Seaweed sample preparation

The brown seaweed samples were rinsed under running water to remove any surface impurities such as sand and sediments. The seaweed samples were then frozen overnight at −80 °C before being subjected to vacuum freeze-drying for 72 h (Dynavac designed FD3, Hingham, MA, USA). The freeze-dried samples were ground into fine powder using a grinder (Cuisinart® Spice and Nut Grinder, SG-10 A, Asquith, NSW, Australia) and stored at −20 °C before further extraction and analysis.

2.3. Ultrasonic extraction of phenolic compounds

Ultrasonic extraction of phenolic compounds was performed at the optimized experimental conditions as outlined by Lee et al. (2024). The phenolic extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator. The concentrated extracts were frozen at −80 °C for 24 h and vacuum freeze-dried for 72 h. The resulting powders were stored at −20 °C before further analysis.

2.3.1. Characterization of phenolic extract

The total phenolic content (TPC), total flavonoid content (TFC), and total phlorotannin content (TPhC) of the phenolic extracts were determined according to the method by Lee et al. (2024). The results of TPC, TFC and TPhC were expressed as mean gallic acid equivalent (GAE ± standard deviation (SD)), mean quercetin equivalent (QE ± SD), and mean phloroglucinol equivalent (PGE ± SD), respectively. All analyses were conducted in triplicate using 1 mg/mL of the dried seaweed phenolic powder dissolved in 70 % ethanol.

2.4. Extraction of sulphated polysaccharide

Extraction of sulphated polysaccharide was carried out according to the method by Xie et al. (2024) with some modifications. The dried seaweed powders were first stirred in 80 % ethanol at 1:20 solid-to-solvent ratio for 24 h for defatting, deproteinization, depigmentation, and removal of low molecular weight phenolic and other compounds. This pretreatment process was repeated thrice. The mixture was then filtered using a polytetrafluoroethylene (PTFE) membrane filter, and the residues were collected for further treatment. Extraction of polysaccharides was performed by heating the residues with 0.1 M HCl (1:20 solid-to-solvent ratio) at 60 °C for 4 h with continuous stirring. This process was repeated three times for complete extraction. After cooling down the mixture to room temperature (RT), an equal volume of 2 % calcium chloride was added to precipitate alginate. The mixture was left to stand overnight at 4 °C and was again filtered with a PTFE membrane. The resulting supernatant was collected, and absolute ethanol was added to precipitate the polysaccharides overnight. The precipitate was collected by centrifugation at 8000 rpm for 5 min. Rotary evaporation was carried out to remove residual ethanol in the precipitate. The precipitate was subsequently dissolved in water, frozen overnight at −80 °C and freeze-dried for 120 h. The resulting powder was stored at −20 °C for further analysis.

2.4.1. Characterization of polysaccharide

2.4.1.1. Chemical composition determination. The total carbohydrate content was determined by the phenol-sulfuric acid method using glucose as the standard (DuBois et al., 1956). The Bradford method using bovine serum albumin as the standard and TPC using gallic acid as the standard were used to measure the protein and phenolic content, respectively (Bradford, 1976; Singleton & Rossi, 1965). The uronic acid content, using D-glucuronic acid as the standard, was determined using the carbazole-sulfuric acid method as outlined by Taylor and Buchanan-Smith (1992). Sulphate content was measured using the barium chloride-gelatine method with potassium sulphate as the standard (Kawai et al., 1969). All analyses were carried out in triplicate, and results were expressed as mean ± SD.

2.4.1.2. Monosaccharide composition. Polysaccharide samples (2 mg) were hydrolysed in 2 mL of 4 M trifluoroacetic acid under a nitrogen atmosphere followed by an 8-h incubation at 100 °C in a water bath. After cooling down to RT, the hydrolysed samples were neutralized to pH 7. Derivatisation of the hydrolysed sugars and monosaccharide standards (arabinose (Ara), fucose (Fuc), galactose (Gal), galacturonic acid (GalUA), glucose (Glc), glucuronic acid (GlcUA), mannose (Man), rhamnose (Rhm), and xylose (Xyl)) was carried out using 1-phenyl-3-methyl-5-pyrazolone (PMP) according to the method outlined by Xie et al. (2024). Briefly, the hydrolysed sugars and monosaccharide samples were dissolved in 500 μL of 0.3 M NaOH aqueous solution and 500 μL 0.5 M PMP methanolic solution. The mixtures were then incubated in a 70 °C water bath for 30 min. The resulting solution was neutralized with 0.3 M HCl after cooling down to RT. Then, 10 mL of chloroform was added to each of the solution and mixed vigorously. The upper aqueous layer was filtered using a 0.45 μm syringe filter and subjected to high-performance liquid chromatography (HPLC) (Agilent 1200 series, Agilent Technologies, CA, USA) using a Synergi Hydro-RP C18 reversed-phase column (Ø 250 × 4.6 mm, 4 μm, Phenomenex, Lane Cove, NSW, Australia) connected to a diode array detector (DAD). The separation of the derivatised sugars was carried out using phosphate buffer and acetonitrile solution (83:17, *v/v*, pH = 6.7) as the mobile phase at a

flow rate of 1.0 mL/min. The injection volume was 20 μL, and the detection wavelength was set at 250 nm.

2.4.1.3. Fourier transform infrared (FT-IR) spectroscopy analysis. The FT-IR spectroscopy analysis was performed using an Alpha II compact FT-IR spectrometer (Bruker, MA, USA). Each freeze-dried sample (5 mg) was pressed onto the diamond crystal and subsequently analysed within the range of 4000–400 cm^{-1} at a scan rate of 16 scans min^{-1}.

2.5. Antioxidant activity study

2.5.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity

The determination of DPPH radical scavenging activity was carried out according to Silva et al. (2024) with some modifications. Briefly, DPPH solution (0.1 mM) was prepared in 100 % ethanol. The assay was carried out by adding 50 μL of the DPPH solution to all the experimental conditions. After 30 min of incubation at RT in the dark, the absorbance was measured at λ 517 nm. The antioxidant activity was calculated as DPPH radical scavenging activity (%) according to the formula: $(A_0 - A_1)/A_0 \times 100\%$, where A_0 is the control absorbance, and A_1 is the absorbance of the sample.

2.5.2. 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The ABTS radical scavenging activity was measured using the method outlined by Borazjani et al. (2017) with some modifications. The ABTS radical cation ($ABTS^{\bullet+}$) was produced by adding 22 μL of 140 mM potassium persulfate ($K_2S_2O_8$) solution to 1.25 mL of 7 mM ABTS solution. The mixture was left in the dark at RT for 16 h. Before use, the dye solution was diluted with methanol to an absorbance of 0.7 at λ 734 nm. The assay was carried out by adding 100 μL of the ABTS solution to each sample, followed by a 6-min incubation period at RT in the dark. The absorbance of the samples was measured at λ 734 nm. The antioxidant activity was calculated as ABTS radical scavenging activity (%) according to the formula: $(A_0 - A_1)/A_0 \times 100\%$, where A_0 is the control absorbance, and A_1 is the absorbance of the sample.

2.5.3. Adapted checkerboard assay

To investigate the antioxidant interaction between polysaccharides and phenolic extracts of brown seaweed, binary mixtures were prepared using the checkerboard assay method as outlined by Silva et al. (2024). The range of concentrations tested in combination was established by firstly performing preliminary DPPH and ABTS assays for each extract. All assays were carried out in triplicate for each combination.

2.5.4. Data analysis by SynergyFinder plus

The assessment of the antioxidant interaction was conducted using the SynergyFinder Plus software. All the results from DPPH and ABTS assays were calculated following the description in Sections 2.5.1 and 2.5.2. The data were processed according to the instructions on the SynergyFinder Plus website. All results presented in this work were generated from the SynergyFinder Plus report.

2.6. Molecular docking study

Molecular docking between fucoidan and phenolic compounds (gallic acid, catechin, and phloroglucinol) was conducted according to the methods outlined by Yadav et al. (2018). The 3D structures of catechin (ID: 73160), gallic acid (ID: 370), and phloroglucinol (ID: 359) were downloaded from PubChem. The 3D structure of fucoidan was drawn and optimized using the Avogadro Software Tool. All downloaded and constructed molecules were converted into .pdb file using PyMOL version 2.5.1. The molecules were then imported into AutoDockTools-1.5.6 (ADT-1.5.6) for docking preparations, which includes charge assignment (Gasteiger charges) and rotatable bond setting. This preparation utilised the AutoDock 4.0 semi-empirical free energy force field. The docking grid map was also constructed using ADT-1.5.6 (Table S1). The molecular docking procedure itself was carried out using ADT-1.5.6-Vina. In the docking study, the fucoidan polymer was considered the rigid 'receptor' body, and the phenolic compounds were fully flexible ligands. The docking results were visualized and analysed using Discovery Studio Visualizer version 4.0. The Cartesian coordinates for the complexes were included in Table S2.

2.7. Complex preparation

Complex preparation of the commercial fucoidan standard (Fuc_{std}) and phenolic compounds (gallic acid, catechin, and phloroglucinol) was carried out according to the method outlined by Gao et al. (2022) with some minor modifications. Briefly, 200 mg of fucoidan standard was dissolved in 20 mL of 0.1 M phosphate buffer solution, and 20 mg of catechin, gallic acid and phloroglucinol were dissolved in 1 mL of ethanol respectively. The prepared solutions were vortexed and ultrasonicated for 5 min to obtain a homogeneous solution. The phenolic solution was then added dropwise into the fucoidan solution while stirring at 500 rpm. The resulting mixture was stirred continuously for 2 h at 500 rpm to obtain the complex. The resulting mixture were freeze-dried and stored at −20 °C for further analysis.

2.7.1. Fourier transform infrared (FT-IR) spectroscopy analysis

The FTIR spectrum of Fuc_{std}, phenolic compounds, physical mixture of fucoidan and phenolic compound, as well as the complex formed above were analysed using FTIR according to the method described in Section 2.4.1.3.

2.7.2. Dynamic light scattering (DLS)

The particle size distribution of the complex formed was measured by a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, Malvern Instruments Co., Ltd., Worcestershire, UK).

2.7.3. Differential scanning calorimetry (DSC)

Thermo transition of approximately 2 mg of samples in a hermetic aluminium crucible was characterized using a DSC 8500 Double Furnace HyperDsc (PerkinElmer, Connecticut, USA). The DSC curve of each sample was established in the range of 25–200 °C at a heating rate of 10 °Cmin^{-1} under flowing nitrogen (40 mLmin^{-1}).

2.8. Statistical analysis

Data were expressed as mean value ± SD of triplicates. Where applicable, statistical analysis was performed using one-way analysis of variance (ANOVA), followed by post-hoc tests with Minitab 19.0 Software for Windows. Graphs were generated using GraphPad Prism software (Version 9.5.1, San Diego, USA) and OriginPro 2024 Software (OriginLab Corp., Northampton, MA).

3. Results and discussion

3.1. Characterization of phenolic extract

Brown seaweeds are rich in phenolic compounds which confer multiple health benefits such as antioxidant properties that mitigate oxidative stress in the body. We extracted phenolic compounds from two Australian brown seaweed species, *Cystophora siliquosa* and *Phyllospora comosa*. Ultrasonication was utilised for extraction as it has superior extraction efficiency as compared to conventional extraction methods such as overnight stirring in an organic solvent (Lee et al., 2024). The phenolic contents of the seaweed phenolic extracts are presented in Table 1. The varying phenolic content across different seaweed species stems from multiple factors, such as species variations, plant growth stage, size, light exposure etc. (Generalić Mekinić et al., 2019).

Table 1
Phenolic content of brown seaweed phenolic extract.

	TPC (mg GAE/g)	TPhC (mg PGE/g)	TFC (mg QE/g)	TCT (mg CE/g)
CS-PE	39.89 ± 1.11	37.05 ± 1.36	0.25 ± 0.02	14.78 ± 0.10
PC-PE	11.53 ± 0.43	6.46 ± 0.46	ND	ND

The data were presented as mean ± SD (n = 3). Abbreviations: CS-PE: *Cystophora siliquosa* phenolic extract; PC-PS: *Phyllospora comosa* phenolic extract; CE (catechin equivalents), GAE (gallic acid equivalents), PGE (phloroglucinol equivalents), QE (quercetin equivalents), TCT (total condensed tannin), TFC (total flavonoid content), TPC (total phenolic content), and TPhC (total phlorotannin content). ND, not detected.

C. siliquosa has a higher phenolic content, measured as TPC, TPhC, TFC or TCT, compared to *P. comosa*, which is in line with our previous study (Lee et al., 2024).

3.2. Characterization of polysaccharide

3.2.1. Chemical characterization of polysaccharide

Polysaccharides extracted from brown seaweed, primarily fucoidan and laminarin, were characterized for their chemical composition (Table 2). These polysaccharides are well known for their biological activities, such as antioxidant and anticancer properties (Sanniyasi et al., 2023). The total carbohydrate content of CS-PS and PC-PS as glucose equivalents are similar, at 62.48 ± 0.37 and 66.54 ± 0.35 % of dry weight, respectively. Sulphate content in CS-PS (32.43 ± 0.42 %) is significantly higher compared to PC-PS (16.29 ± 0.34 %), the commercial fucoidan standard from *Undaria pinnatifida* (Fuc_{std}, 17.10 ± 0.38 %) and from other species such as *Fucus vesiculosus* (20.10 %) and *Ascophyllum nodosum*, (30.7 %). This elevated sulphate content suggests stronger bioactivity from CS-PS as previous research has shown correlation between sulphate content and bioactivity such as antioxidant activity (Amin et al., 2024; Hifney et al., 2016; Koh et al., 2019; Palanisamy et al., 2017). The uronic acid contents of CS-PS (6.26 ± 0.02 %) and PC-PS (10.06 ± 0.47 %) are higher than that of Fuc_{std} (2.43 ± 0.10 %) and those from other species (3.40–4.06 %) (Koh et al., 2019; Manikandan et al., 2020; Palanisamy et al., 2017). Seasonal variation, extraction method and species-specific factors are crucial factors that drive the diverse structure and composition of marine polysaccharides, explaining the disparity in the polysaccharide compositions seen above (Fletcher et al., 2017). As expected, protein and phenolic content of the extracted polysaccharides were low as a result of the 80 % ethanol pretreatment in the extraction protocol, aligning with other findings (Xie et al., 2024). Following analysis of the polysaccharide chemical composition, their monosaccharide composition further highlights key structural differences among the seaweeds. The dominance of fucose in CS-PS (45.47 %), PC-PS (36.04 %) and Fuc_{std} (53.18 %) along with varying amounts of galactose, mannose, and xylose reflects a typical fucoidan structure (Amin et al., 2024). The galactose content in Fuc_{std} is higher than in the extracted polysaccharide from CS and PC, at 41.46 %. This observation is similar to previous research that reported a 1:1 ratio of fucose to galactose in fucoidan extracted from *Undaria pinnatifida* (Koh et al., 2019).

3.2.2. FT-IR spectroscopy

Fig. 1 shows the FTIR spectrum of the extracted polysaccharides, where specific absorption bands were identified to elucidate the structural characteristics. The bands observed at 3380–3430 cm^{-1} representing O—H stretching vibration and at 2900 cm^{-1} representing C—H stretching vibration, are common features in polysaccharides. The peak at ~1620 cm^{-1} corresponds to the carboxylate O-C-O asymmetric stretching vibration, indicating the presence of uronic acid (Hifney et al., 2016), thus corroborating the compositional results in Table 2. The stretching peaks near 1400 cm^{-1} correspond to the asymmetric bending vibrations of -CH_3 (Khalafu et al., 2017). The signal at 1237 cm^{-1} (PC-PS), 1250 cm^{-1} (CS-PS), and 1224 cm^{-1} (Fuc_{std}) represent the asymmetric stretching of S═O, which is a characteristic peak in sulphated polysaccharide in marine algae, especially in fucoidan (Manikandan et al., 2020). To further confirm the presence of sulphate groups in the extracted polysaccharide, the sharp peak at 824 cm^{-1} (C-S-O) indicates sulphation at the equatorial position (C-2 and C-3) whereby fucose is bonded to sulphate ester (Manikandan et al., 2020; Yuan & Macquarrie, 2015). Fuc_{std} exhibited peaks at 835 cm^{-1} and 817 cm^{-1}, indicating the presence of sulphate group at both axial (C-4) and equatorial position (Yuan & Macquarrie, 2015).The stronger intensity of peaks at 1250 cm^{-1} and 824 cm^{-1} in CS-PS, compared to corresponding peaks in PC-PS indicates a higher degree of sulfation in CS-PS which correlates well with the results in Table 2. The FTIR analysis validated the compositional analysis (Section 3.2.1), and highlighted key structural uronic acid and sulphate groups features, which can provide bioactivity for the polysaccharides.

3.3. Antioxidant activity of phenolic and polysaccharide extract

The DPPH and ABTS scavenging assays were used to determine the antioxidant activity of the brown seaweed phenolics and polysaccharides. As shown in Fig. 2, the phenolic extracts have higher DPPH radical scavenging activity than the polysaccharide extracts and Fuc_{std} on weight basis. This result highlights the greater radical scavenging ability of phenolic compounds, which can be attributed to aromatic hydroxyl group entities, which enable proton donation and stabilization of the resulting antioxidant free radical (Zeb, 2020). In contrast, the radical scavenging activity of polysaccharides is dependent on their molecular weight and sulphate content (Li et al., 2008). CS-PS showed higher DPPH radical scavenging activity than PC-PS that can be linked to its higher sulphate content (Table 2). According to Wang et al. (2010), sulphate groups can enhance the hydrogen donating ability by activating the hydrogen atom on the anomeric carbon in fucoidan.

A similar trend was observed in the reduction of the ABTS radical in the more polar solution environment of the ABTS assay, whereby the phenolic extracts once again demonstrated superior antioxidant activity compared to the polysaccharide extracts and Fuc_{std}. Taken together, the seaweed phenolics possess a stronger radical scavenging activity than

Table 2
Proximate composition of seaweed polysaccharide extract and $Fuc_{std.}$

	Total Carbohydrate Content (%)	Total Uronic Acid Content (%)	Total Sulphate Content (%)	Total Protein Content (%)	Total Phenolic Content (%)	Monosaccharide Composition (%)								
						Man	Rha	GlcUA	GalUA	Glc	Gal	Xyl	Ara	Fuc
CS-PS	62.48 ± 0.37	6.26 ± 0.02	32.42 ± 0.42	n.d.	0.90 ± 0.02	20.06	0.99	1.41	–	3.45	24.31	4.30	–	45.47
PC-PS	66.54 ± 0.35	10.06 ± 0.47	16.29 ± 0.34	0.20 ± 0.06	1.01 ± 0.03	28.62	–	5.20	–	2.60	7.92	17.26	2.36	36.04
Fuc_{std}	53.51 ± 0.28	2.43 ± 0.10	17.10 ± 0.38	n.d.	n.d.	5.36	–	–	–	–	41.46	–	–	53.18

The data were presented as mean ± standard deviation (n = 3). Total Carbohydrate Content (%) was expressed as glucose equivalent. Abbreviations: CS-PS: *Cystophora siliquosa* polysaccharide; PC-PS: *Phyllospora comosa* polysaccharide; Fuc_{std}: Fucoidan standard from *Undaria pinnatifida*; Ara: arabinose; Fuc: fucose; Gal: galactose; GalUA: galacturonic acid: Glc; GlcUA: glucuronic acid; Man: mannose; Rhm: rhamnose; Xyl: xylose.

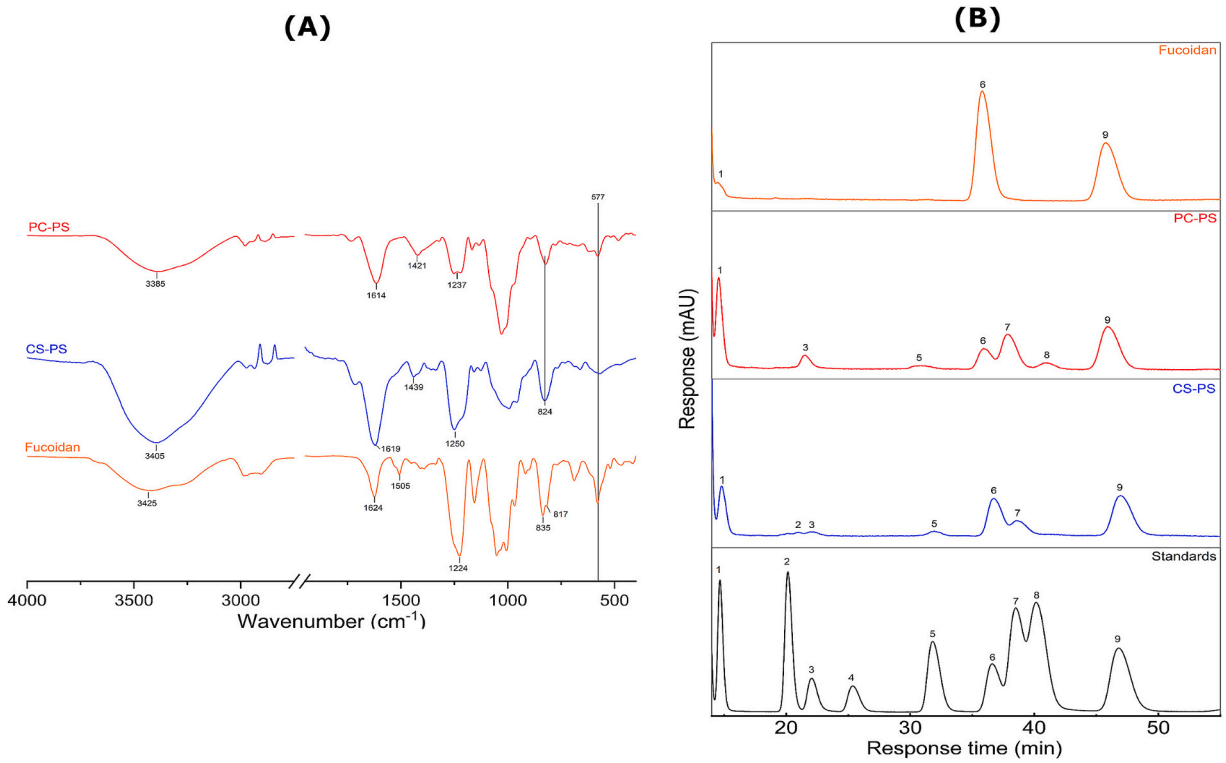

Fig. 1. FTIR spectra (A) and HPLC-DAD analysis of monosaccharide composition (1-Man: mannose; 2-Rha: Rhamnose; 3-GlcUA: glucuronic acid; 4-GalUA: galacturonic acid; 5-Glc: glucose; 6-Gal: galactose; 7-Xyl: xylose; 8-Ara: arabinose; 9-Fuc: fucose) of CS-PS, PC-PS and Fuc_{std}. CS-PS: *Cystophora siliquosa* polysaccharide; PC-PS: *Phyllospora comosa* polysaccharide; Fuc_{std}: Fucoidan standard from *Undaria pinnatifida* (B).

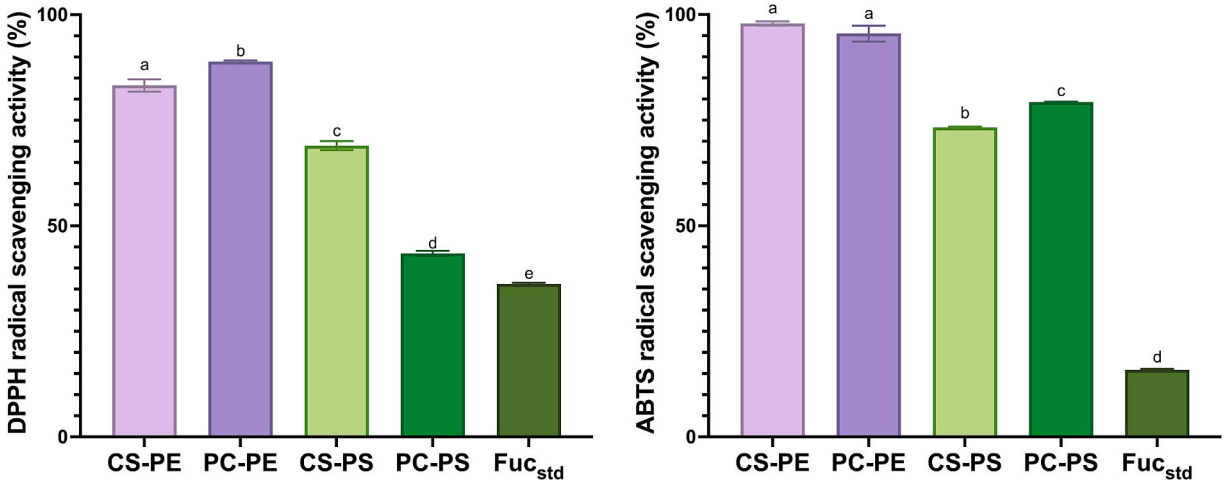

Fig. 2. DPPH and ABTS radical scavenging activity of Fuc_{std}, seaweed polysaccharide and phenolic extract (5 mg/mL). Results are expressed by mean ± standard deviation ($n = 3$). Abbreviations: CS-PE: *Cystophora siliquosa* phenolic extract; PC-PE: *Phyllospora comosa* phenolic extract; CS-PS: *Cystophora siliquosa* polysaccharide; PC-PS: *Phyllospora comosa* polysaccharide; Fuc_{std}: Fucoidan standard from *Undaria pinnatifida*.

the seaweed polysaccharides that can be related to their structures.

3.4. Antioxidant interaction between polysaccharide and phenolic extract

Phenolic compounds can interact with polysaccharides, potentially altering their antioxidant activity with either synergistic or antagonistic effects. To investigate this, a checkerboard assay was applied to efficiently combine polysaccharide and phenolic extracts at different ratios, followed by evaluation of their antioxidant capacity using the DPPH and ABTS assays. SynergyFinder Plus, an online tool used by Silva et al. (2024), was applied to analyse these interactions mathematically. In this study, we chose to use the Zero-Interaction Potency (ZIP) model for its efficient calculation, enhanced flexibility, and low false positive rates (Ma et al., 2024).

The results presented in Figs 3 a and b show that the combination of polysaccharide and phenolic (extracts and standards) predominantly exhibited an antagonistic effect, whereby the observed antioxidant activity is lower than the sum of individual antioxidant activities of each compound in the DPPH and ABTS radical scavenging activity. This effect is more prominent at lower phenolic concentrations. A slight synergistic effect, whereby the observed antioxidant activity exceeds the combined individual antioxidant activities of each compound, is observed between

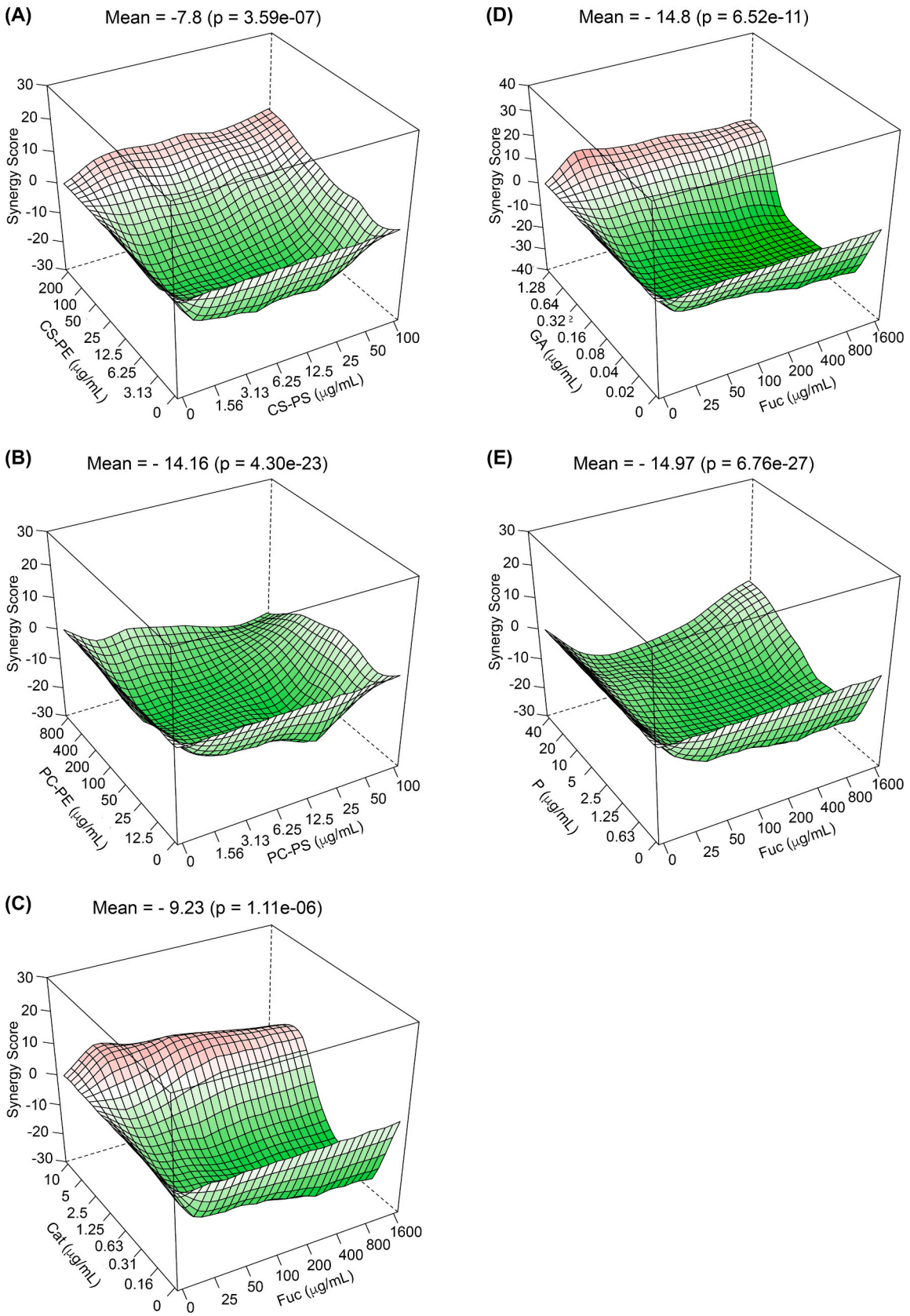

Fig. 3. Fig. 3a DPPH radical scavenging synergy map of binary mixture. Polysaccharide and phenolic extract from *Cystophora siliquosa* (A), *Phyllospora comosa* (B) and Fuc_{std} with catechin (C), gallic acid (D) and phloroglucinol (E) in the DPPH assay. The concentration range as μg or mg per mL of each entity pair indicated in the plots. Synergic interactions are represented with positive scores (red area), antagonistic interactions with negative scores (green area), and additive interactions with scores around zero (white area) in the synergy map. CS-PE: *Cystophora siliquosa* phenolic extract; PC-PE: *Phyllospora comosa* phenolic extract; Cat: Catechin; GA: gallic acid; P: Phloroglucinol; CS-PS: *Cystophora siliquosa* polysaccharide; PC-PS: *Phyllospora comosa* polysaccharide; Fuc_{std}: Fucoidan standard from *Undaria pinnatifida.*

(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.). Fig. 3b. ABTS radical scavenging synergy map of binary mixture. Polysaccharide and phenolic extract from *Cystophora siliquosa* (A), *Phyllospora comosa* (B) and Fuc_{std} with catechin (C), gallic acid (D) and phloroglucinol (E) in the ABTS assay. The concentration range as μg or mg per mL of each entity pair indicated in the plots. Synergic interactions are represented with positive scores (red area), antagonistic interactions with negative scores (green area), and additive interactions with scores around zero (white area) in the synergy map. CS-PE: *Cystophora siliquosa* phenolic extract; PC-PE: *Phyllospora comosa* phenolic extract; Cat: Catechin; GA: gallic acid; P: Phloroglucinol; CS-PS: *Cystophora siliquosa* polysaccharide; PC-PS: *Phyllospora comosa* polysaccharide; Fuc_{std}: Fucoidan standard from *Undaria pinnatifida.* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fuc_{std} and phloroglucinol in the ABTS assay. Our findings are in line with Silva et al. (2024), who suggests that presence of polysaccharides may impede phenolic compounds from scavenging free radicals. Conversely, Mercado-Mercado et al. (2020) and Mihai et al. (2023) showed that the combination of polysaccharide and phenolic compounds can boost their antioxidant capacity, possibly due to the protective effect of polysaccharide against phenolic compound oxidation. Interestingly, Domínguez Avila et al. (2018) noted the synergistic or antagonistic effect of pectin addition is dependent on the type of phenolic compound used, indicating interactions between the two. These studies underscore the importance of structural compatibility and the relative ratios of polysaccharide and phenolic compounds in governing their interaction.

The antagonistic effect observed in our study could be due to the ability of polysaccharide to trap phenolic compounds or complex formation between polysaccharide and phenolic compounds (Fernandes & Coimbra, 2023; Silva et al., 2024). Previous studies have highlighted non-covalent interactions such as hydrogen bonding and *Van der Waals* forces between polysaccharide and phenolic compounds. Fucoidan, a primary polysaccharide in brown seaweed, is particularly interesting due to its unique structural features which include high sulphate content, the presence of uronic acid and a branched backbone. These features enhance its mechanical flexibility, allowing it to interact with other biomolecules such as phenolic compounds (Chadwick et al., 2025). Phenolic compounds such as gallic acid, catechin and phloroglucinol that are commonly found in brown seaweed contain benzene ring(s) and multiple -OH groups that can non-covalently interact with the sulphate and hydroxyl groups on fucoidan. This could potentially lead to entrapment of phenolic compounds or the formation of a fucoidan-phenolic complex, decreasing the ability of phenolic compounds to scavenge free radicals. The structural flexibility of fucoidan enhances its ability to potentially trap the phenolic compound, further reinforcing the possibility of complex formation and in explaining the antagonistic effect observed.

To further investigate this possible interaction, we used molecular docking to elucidate the possible interaction between fucoidan and phenolic compounds followed by experimental validation by preparing complexes of Fuc_{std} and pure phenolic compounds, as described in Section 2.7.

3.5. Characterization of polysaccharide-phenolic mixture

3.5.1. Molecular docking analysis

In silico molecular docking studies take advantage of computational models to illustrate the possible interaction between fucoidan and phenolic compounds. Fig. 4 illustrates the modelled interaction between fucoidan and phenolic compounds, showing their lowest binding affinities. The phenolic compounds have varying affinities towards fucoidan, with the binding affinity ranked as follows: catechin (-4 kcalmol^{-1}) > gallic acid (-3 kcalmol^{-1}) > phloroglucinol (-2.5 kcalmol^{-1}). These values suggest that the interaction between fucoidan and catechin is most favourable. Hydrogen bonds were observed in all three interactions, aligning with previous studies that identified hydrogen bonding as the main driving force between polysaccharides and phenolic compounds (Fernandes et al., 2014; Liu et al., 2018). This suggests that hydrogen bonding plays a dominant role in complex stabilization. Furthermore, the presence of sulphate group in fucoidan leads to the formation of π-sulphur interactions with the benzene ring in catechin and phloroglucinol, further enhancing the stability of the complex formed. Overall, the docking studies suggest the possible complex formation between fucoidan and phenolic compounds. However, these results need to be interpreted with caution, as further experimental validation is required to confirm these in silico findings.

3.5.2. FT-IR analysis

FT-IR spectroscopy was conducted on Fuc_{std}, catechin, gallic acid, phloroglucinol, and their respective physical mixtures (FCM, FGAM, FPM) and complexes (FCC, FGAC, FPC) to assess complex formation (Fig. 5a). Catechin, gallic acid and phloroglucinol exhibited characteristic absorption bands at 1000–1600 cm^{-1}, consistent with previous reports (Khan et al., 2022; Liu et al. 2014; Santoso et al., 2021). Notable changes were observed in the FCC spectrum when compared with the FCM spectrum: peak attenuation at 1521 cm^{-1} and 1463 cm^{-1}; peak shifting from 1383 cm^{-1} to 1386 cm^{-1}, and 1147 cm^{-1} to 1143 cm^{-1}; the disappearance of catechin's characteristic peaks at 1079 cm^{-1}, 1026 cm^{-1} and 764 cm^{-1}. Similarly, FPC showed characteristic phloroglucinol peak (1507 cm^{-1}), with peak shifting and attenuation at the following wavelengths: 1152 cm^{-1} to 1155 cm^{-1}, 1000 cm^{-1} to 1004 cm^{-1}, 813 cm^{-1} to 817 cm^{-1}, 517 cm^{-1} to 520 cm^{-1}. The shifting of peaks in FCC and FPC indicates changes in bond length and angle due to hydrogen bond formation between fucoidan and the phenolic compounds (Liu et al., 2018). Reduced peak intensity further supports hydrogen bond formation, as noted by previous studies (Guo et al., 2018; Liu et al., 2018; Peng et al., 2023). Taken together, these changes indicate complex formation occurs between fucoidan and catechin and phloroglucinol. The formation of complexes between catechin and phloroglucinol and other polysaccharides, such as chitosan, cellulose, and pectin has been reported in literature (Khan et al., 2022; Liu et al., 2018). Thus, the results from our study align with previous studies on the formation of a complex between fucoidan and catechin and phloroglucinol.

In contrast, no spectral changes were observed for the FGAC, suggesting minimal or no interaction between fucoidan and gallic acid. This is in line with the chemical behavior of gallic acid, whereby previous studies showed non-covalent interactions between gallic acid with neutral-charged polysaccharide such as starch, chitosan, cyclodextrin, and inulin (da Rosa et al., 2013; Han et al., 2020; Peng et al., 2023; Sabando et al., 2022; Zarandona et al., 2020), despite the fact that gallic acid contain hydrogen bond capable OH groups. However, these proximate OH groups are likely to engage in intramolecular hydrogen bonding between themselves, making them unavailable for external entities. However, gallic acid can interact with negatively charged polysaccharide such as pectin, but this interaction is covalent, mediated by an enzyme-driven reaction where gallic acid is oxidized to quinone, followed by esterification with the hydroxyl group of the sugar in the polysaccharide (Huang et al., 2021; Liu et al., 2021; Xue et al., 2024; Zhang et al., 2020). Based on current literature findings, the non-oxidized form of gallic acid in this study may lack interaction with fucoidan. This contrasts with the molecular docking results (Section 3.5.1), where a single hydrogen bond was predicted between gallic acid and fucoidan, while multiple interactions were predicted for catechin and phloroglucinol. Thus, the predicted single hydrogen bond may not be sufficient to form a stable complex with fucoidan, particularly in the absence of other stabilizing interactions. The discrepancies noted here show the limitations of the AutoDock Vina scoring function, which may lead to the overestimation of the contribution of a single hydrogen bond.

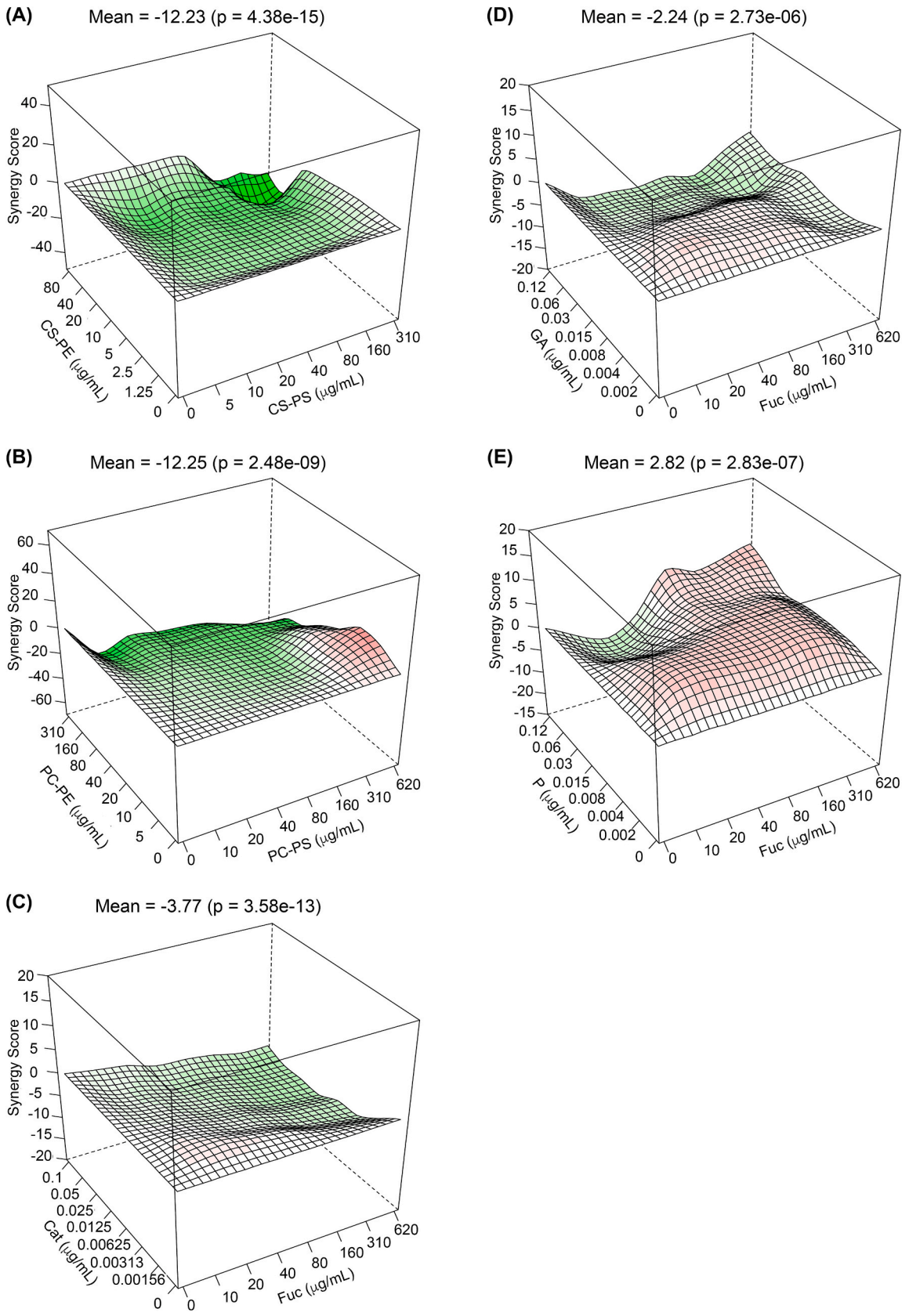

Fig. 3. (*continued*).

Therefore, it is critical to interpret in silico results with caution and to verify the findings with experimental evidence to draw accurate conclusions.

These results highlight the significance of the phenolic structural role in mediating their interaction with fucoidan. The presence of heterocyclic structure in catechin and the compact symmetric structure of

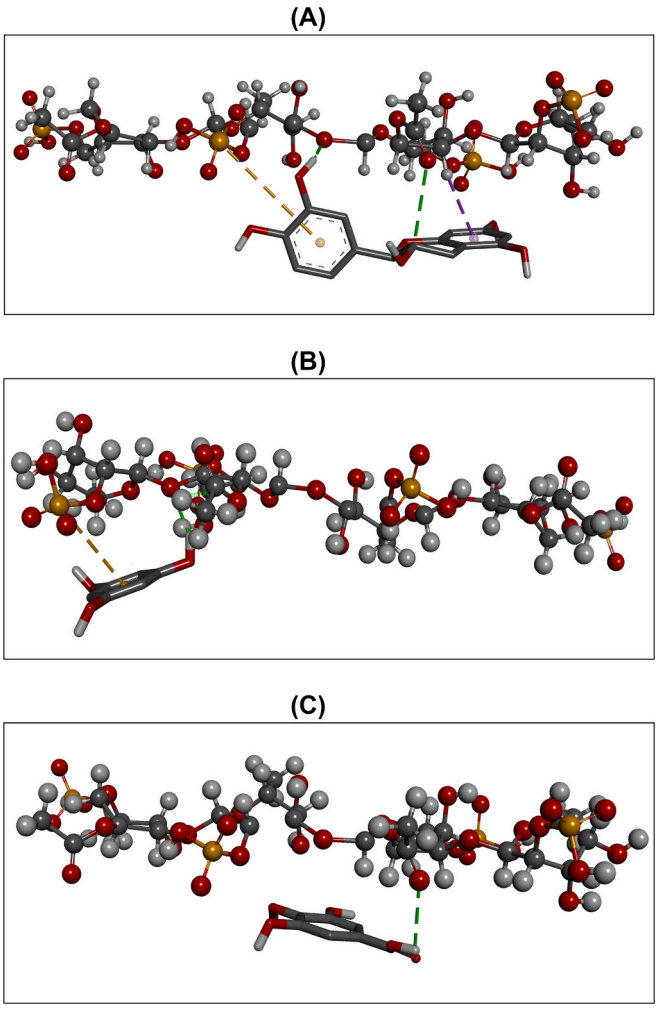

Fig. 4. Molecular docking study. Lowest binding energy conformations of fucoidan and (A) catechin, (B) phloroglucinol, (C) gallic acid obtained by in-silico docking calculations.

phloroglucinol may contribute to complex stabilization effect whereas gallic acid's planar structure and lack of oxidation may limit its interaction with fucoidan.

3.5.3. Dynamic light scattering (DLS)

DLS analysis was performed to determine the particle size distribution of the complex formed (Fig. 5b). No distinct differences in the particle size of FGAM and FGAC were observed, supporting the FTIR findings whereby no complex formation was noted with gallic acid. The particle size of FCC and FPC were determined to be 230.9 d.nm and 349.7 d.nm, respectively, which differed significantly from their respective mixtures, in which two size peaks were observed, indicating heterogenous distribution of particles from the two un-complexed components. The observed differences in particle size suggest complexation between fucoidan and the phenolic compounds occurs, further corroborating the FTIR findings.

The reduction in particle size of the complex may result from the tighter packing of molecules due to integration of phenolic compounds into the fucoidan structure via hydrogen bonding and other non-covalent interactions. However, this observation contrasts with other studies which noted an increase in particle size during polysaccharide-phenolic interaction due to colloidal particle aggregation (Deepika et al., 2019; Peng et al., 2023). These discrepancies may be explained by the differences in molecular structure and chemical properties of phenolic compounds and polysaccharides involved. For instance, Tudorache and Bordenave (2019) found that the complexity and structure of phenolic compounds such as number of hydrogen bonding groups significantly influence the change in particle size upon complexation. Additionally, Dong et al. (2023) reported increased particle size in anthocyanin complexes formed between oat beta glucan and konjac glucomannan but decreased complex size when formed with xanthan gum. This suggests that polysaccharide molecular charge could influence the particle size of the complex.

In the case of fucoidan, its negative charge and branched backbone may facilitate the formation of a more compact structure with catechin and phloroglucinol. The mechanical flexibility of fucoidan allows it to bring the molecules closer together by aligning its functional groups, such as hydroxyl and sulphate groups with phenolic compound's benzene rings and hydroxyl groups (Chadwick et al., 2025). Furthermore, the docking results showed π-sulphur interaction between the sulphur groups on fucoidan and benzene rings in catechin and phloroglucinol, and this interaction can further stabilize the complex and result in tighter packing of the molecules. In summary, the decrease in particle size for FCC and FPC suggests the formation of a more compact structure due to interactions between fucoidan and the phenolic compounds, further corroborating the findings from the previous section.

3.5.4. Differential scanning calorimetry (DSC)

DSC was used to characterize the thermal properties of the Fuc_{std} and its complexes with catechin, gallic acid and phloroglucinol. After complexation, changes in the polysaccharide thermal properties can indicate possible interactions between molecules at the macroscopic level. Changes in polysaccharide thermal properties are shown in Table 3. For the FGAC, the denaturation temperature and enthalpy are similar to $Fuc_{std,}$ suggesting the absence of complex formation. For the FPC, the denaturation temperature and enthalpy were higher than those of $Fuc_{std,}$ indicating higher thermal stability of FPC. Phloroglucinol has a symmetrical and compact structure, allowing it to effectively form hydrogen bonds with fucoidan without disrupting its internal molecular structure. The integration of phloroglucinol into the fucoidan structure resulted in a more thermally stable complex, which is in line with literature for similar complexes (Guo et al., 2018; Ma et al., 2023).

In contrast, the FCC showed lower denaturation temperature and enthalpy than Fuc_{std}, indicating reduced thermal stability. This result is unexpected, but it may be due to the larger and more complex structure of catechin which contains heterocyclic ring. The formation of hydrogen bonds between catechin and fucoidan may interfere with fucoidan's molecular organization, disrupting its internal hydrogen bond network. This would lead to a disrupted internal conformation of the polysaccharide, causing reduced thermal stability. Similar thermal destabilization has been previously reported for other polysaccharides (Chen et al., 2022; Wang et al., 2018; Wang et al., 2024). The observed difference in thermal stability underscores the importance of phenolic compound's structure in affecting the thermal stability of fucoidan complexes. Compact and symmetric phenolic compound such as phloroglucinol can be effectively integrated into polysaccharide's structure without disrupting its internal molecular structure. In contrast, larger and complex phenolic compound, such as catechin, could destabilize the polysaccharide structure by interfering with its internal bond network. These findings are in line with Li et al. (2024), who reported an increase in thermal stability in curdlan complex formed with curcumin and quercetin but decreased thermal stability in complex formed with chlorogenic acid. This shows that phenolic structure plays a vital role due to differences in binding affinity, hydrogen bond forming ability, and molecular interaction.

4. Conclusion

This study successfully extracted phenolic compounds and polysaccharides from two brown seaweed species, revealing their distinct compositional and structural characteristics. *Cystophora siliquosa*

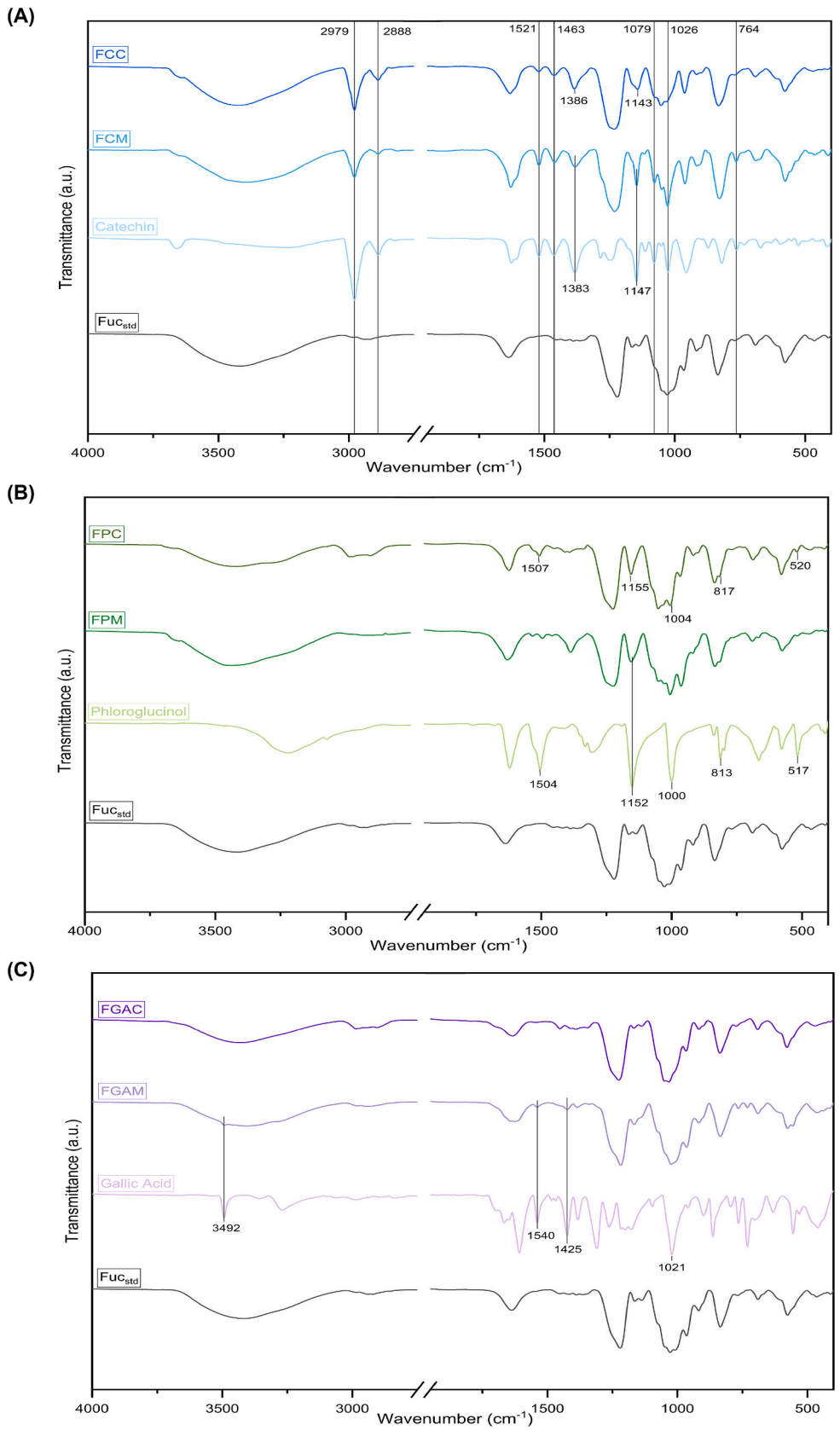

Fig. 5. a FTIR spectra of (A) catechin, FCM, FCC; (B) phloroglucinol, FPM, FPC; (C) gallic acid, FGAM, FGAC. Abbreviations: FGAM: Fucoidan-gallic acid mixture; FGAC: Fucoidan-gallic acid complex; FCM: Fucoidan-catechin mixture; FCC: Fucoidan-catechin complex; FPM: Fucoidan-phloroglucinol mixture; FPC: Fucoidan-phloroglucinol complex. Fig. 5b. Particle size distribution for (A) FCM, FCC; (B) FPM, FPC; (C) FGAM, FGAC. Abbreviations: FGAM: Fucoidan-gallic acid mixture; FGAC: Fucoidan-gallic acid complex; FCM: Fucoidan-catechin mixture; FCC: Fucoidan-catechin complex; FPM: Fucoidan-phloroglucinol mixture; FPC: Fucoidan-phloroglucinol complex.

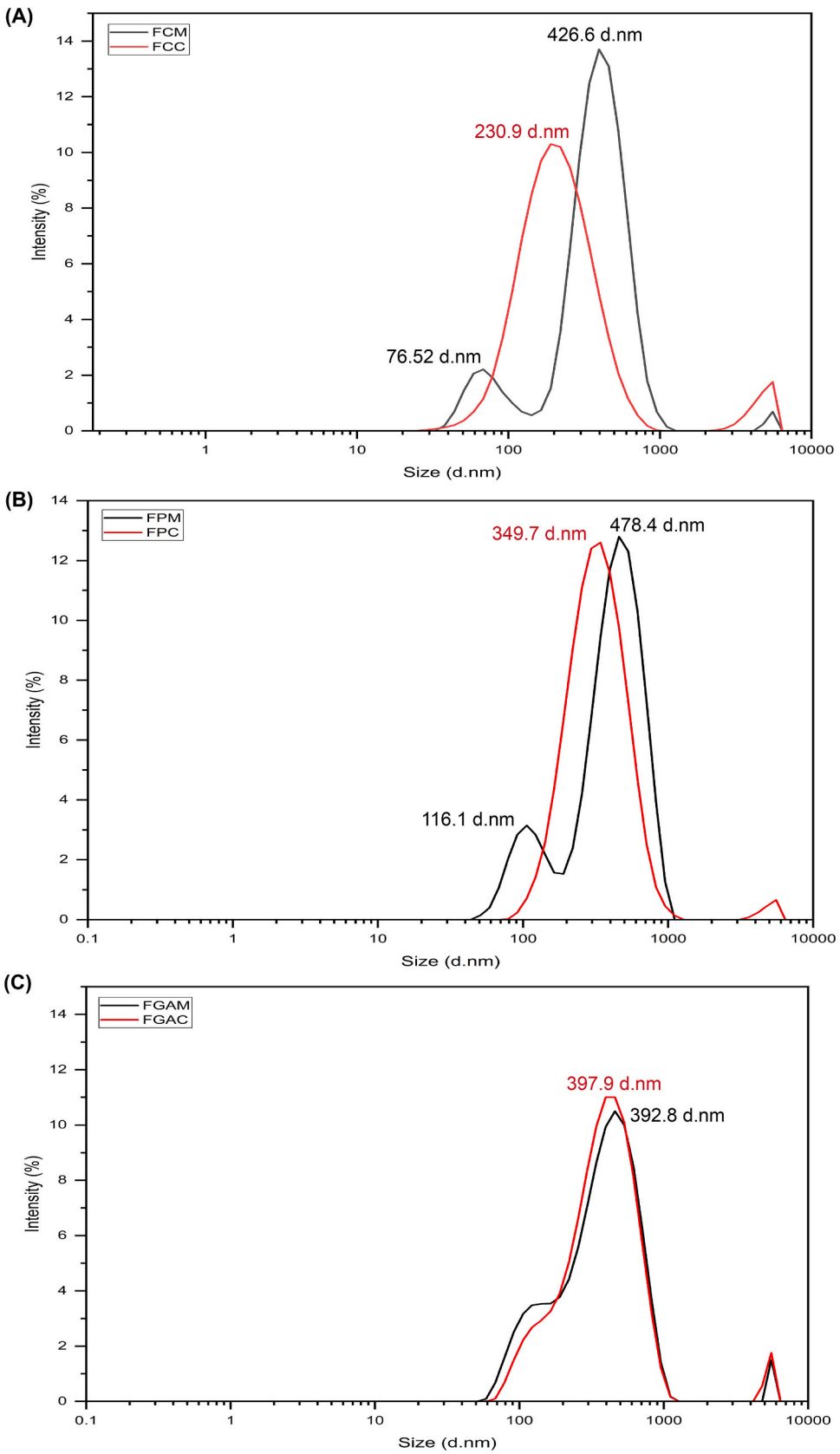

Fig. 5. (*continued*).

presents higher phenolic content, and its polysaccharides present a higher sulphate content, which contributes to their superior antioxidant activity. The combination of phenolic compounds and polysaccharides resulted in an antagonistic effect on antioxidant activity, likely due to complex formation between the two. In silico molecular docking, supported by FTIR, DLS, and DSC studies, showed complexation of fucoidan

Table 3
Thermal transition parameters of Fuc_{std}, FGAC, FCC and FPC.

Samples	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)	ΔH (Jg^{-1})
Fuc_{std}	163.39	181.75	178.65	−267.1465
FGAC	166.49	180.08	179.31	−219.8141
FCC	154.26	158.80	166.90	−222.8220
FPC	183.79	188.08	186.31	−278.9974

Abbreviations: FGAC: Fucoidan-gallic acid complex; FCC: Fucoidan-catechin complex; FPC: Fucoidan-phloroglucinol complex.

with catechin and phloroglucinol but not with gallic acid. The particle size analysis showed that the formation of fucoidan complexes with catechin and phloroglucinol resulted in smaller particle size, reflecting tighter packing of molecules. DSC analysis revealed that complexation with phloroglucinol improved the thermal stability of fucoidan, while complexation with catechin reduced it. The reduced thermal stability of the FCC may result from disruption of fucoidan's molecular structure, due to hydrogen bond formation.

This study thus highlights the importance of structural features as molecular structure, flexibility, and functional groups, which influence the binding affinity and interaction potential between polysaccharide and phenolic compounds. The findings from this study contribute to a deeper understanding of polysaccharide-phenolic interactions in brown seaweed by highlighting their impact on antioxidant activity, thermal stability and structural behavior. The observed antagonistic interaction is critical in functional food and nutraceutical formulation where optimizing the polysaccharide-phenolic interaction is essential to maximise their bioactivity, stability and health benefits. Future research should explore a broader range of phenolic compounds with more diverse structural and functional properties to provide a more comprehensive overview of their interaction with polysaccharide. Additionally, the stability and potential functional effect of these complexes should be investigated using simulated gastrointestinal model and under gut microbiome conditions.

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CRediT authorship contribution statement

Zu Jia Lee: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Cundong Xie:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration, Methodology, Conceptualization. **Ken Ng:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Hafiz A.R. Suleria:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing financial or personal interest that would influence the work in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.143494.

Data availability

Data will be made available on request.

References

Amin, M. N. G., Rosenau, T., & Böhmdorfer, S. (2024). The structure of fucoidan by linkage analysis tailored for fucose in four algae species: Fucus serratus, Fucus evanescens, Fucus vesiculosus and Laminaria hyperborea. *Carbohydrate Polymer Technologies and Applications, 7*, Article 100455. https://doi.org/10.1016/j.carpta.2024.100455

Arata, P. X., Alberghina, J., Confalonieri, V., Errea, M. I., Estevez, J. M., & Ciancia, M. (2017). Sulfated polysaccharides in the freshwater green macroalga Cladophora surera not linked to salinity adaptation. *Frontiers in Plant Science, 8*, 1927. https://doi.org/10.3389/fpls.2017.01927

Borazjani, N. J., Tabarsa, M., You, S., & Rezaei, M. (2017). Improved immunomodulatory and antioxidant properties of unrefined fucoidans from Sargassum angustifolium by hydrolysis. *Journal of Food Science and Technology, 54*(12), 4016–4025. https://doi.org/10.1007/s13197-017-2867-2

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chadwick, M., Carvalho, L. G., Vanegas, C., & Dimartino, S. (2025). A comparative review of alternative Fucoidan extraction techniques from seaweed. *Marine Drugs, 23*(1), 27. https://doi.org/10.3390/md23010027

Chen, N., Feng, Z.-J., Gao, H.-X., He, Q., & Zeng, W.-C. (2022). Effects of phenols with different structure characteristics on properties of potato starch: Action rule and molecular mechanism. *Journal of Food Processing and Preservation, 46*(7), Article e16679. https://doi.org/10.1111/jfpp.16679

Deepika, M. S., Thangam, R., Sheena, T. S., Sasirekha, R., Sivasubramanian, S., Babu, M. D., … Thirumurugan, R. (2019). A novel rutin-fucoidan complex based phytotherapy for cervical cancer through achieving enhanced bioavailability and cancer cell apoptosis. *Biomedicine & Pharmacotherapy, 109*, 1181–1195.

Domínguez Avila, J. A., Villegas Ochoa, M. A., Alvarez Parrilla, E., Montalvo González, E., & González Aguilar, G. A. (2018). Interactions between four common plant-derived phenolic acids and pectin, and its effect on antioxidant capacity. *Journal of Food Measurement and Characterization, 12*, 992–1004.

Dong, R., Tian, J., Huang, Z., Yu, Q., Xie, J., Li, B., Li, C., & Chen, Y. (2023). Intermolecular binding of blueberry anthocyanins with water-soluble polysaccharides: Enhancing their thermostability and antioxidant abilities. *Food Chemistry, 410*, Article 135375. https://doi.org/10.1016/j.foodchem.2022.135375

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & t., & Smith, F.. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356.

Fernandes, A., Brás, N. F., Mateus, N., & de Freitas, V. (2014). Understanding the molecular mechanism of anthocyanin binding to pectin. *Langmuir, 30*(28), 8516–8527.

Fernandes, P. A. R., & Coimbra, M. A. (2023). The antioxidant activity of polysaccharides: A structure-function relationship overview. *Carbohydrate Polymers, 314*, Article 120965. https://doi.org/10.1016/j.carbpol.2023.120965

Fernandes, P. A. R., Le Bourvellec, C., Renard, C. M. G. C., Wessel, D. F., Cardoso, S. M., & Coimbra, M. A. (2020). Interactions of arabinan-rich pectic polysaccharides with polyphenols. *Carbohydrate Polymers, 230*, Article 115644. https://doi.org/10.1016/j.carbpol.2019.115644

Fletcher, H. R., Biller, P., Ross, A. B., & Adams, J. M. M. (2017). The seasonal variation of fucoidan within three species of brown macroalgae. *Algal Research, 22*, 79–86. https://doi.org/10.1016/j.algal.2016.10.015

Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery, 20*(9), 689–709. https://doi.org/10.1038/s41573-021-00233-1

Gao, X., Wang, J., Wang, Y., Liu, S., Dong, K., Wu, J., Wu, X., Shi, D., Wang, F., & Guo, C. (2022). Fucoidan-ferulic acid nanoparticles alleviate cisplatin-induced acute kidney injury by inhibiting the cGAS-STING pathway. *International Journal of Biological Macromolecules, 223*, 1083–1093.

Gao, X., Yin, Y., Liu, S., Dong, K., Wang, J., & Guo, C. (2023). Fucoidan-proanthocyanidins nanoparticles protect against cisplatin-induced acute kidney injury by activating mitophagy and inhibiting mtDNA-cGAS/STING signaling pathway. *International Journal of Biological Macromolecules, 245*, Article 125541.

Generalić Mekinić, I., Skroza, D., Šimat, V., Hamed, I., Čagalj, M., & Popović Perković, Z. (2019). Phenolic content of brown algae (Pheophyceae) species: Extraction, identification, and quantification. *Biomolecules, 9*(6), 244.

Guo, Q., Ma, Q., Xue, Z., Gao, X., & Chen, H. (2018). Studies on the binding characteristics of three polysaccharides with different molecular weight and flavonoids from corn silk (Maydis stigma). *Carbohydrate Polymers, 198*, 581–588.

Han, X., Zhang, M., Zhang, R., Huang, L., Jia, X., Huang, F., & Liu, L. (2020). Physicochemical interactions between rice starch and different polyphenols and structural characterization of their complexes. *LWT, 125*, Article 109227. https://doi.org/10.1016/j.lwt.2020.109227

Hifney, A. F., Fawzy, M. A., Abdel-Gawad, K. M., & Gomaa, M. (2016). Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. *Food Hydrocolloids, 54*, 77–88. https://doi.org/10.1016/j.foodhyd.2015.09.022

Huang, B., Zhang, Z., Ding, N., Wang, B., Zhang, G., & Fei, P. (2021). Investigation of the pectin grafting with gallic acid and propyl gallate and their antioxidant activities, antibacterial activities and fresh keeping performance. *International Journal of Biological Macromolecules, 190*, 343–350. https://doi.org/10.1016/j.ijbiomac.2021.08.219

Jun, J.-Y., Jung, M.-J., Jeong, I.-H., Yamazaki, K., Kawai, Y., & Kim, B.-M. (2018). Antimicrobial and Antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque Bacteria. *Marine Drugs, 16*(9), 301. https://www.mdpi.com/1660-3397/16/9/301.

Kawai, Y., Seno, N., & Anno, K. (1969). A modified method for chondrosulfatase assay. *Analytical Biochemistry, 32*(2), 314–321. https://doi.org/10.1016/0003-2697(69)90091-8

Khalafu, S. H. S., Wan Aida, W. M., Lim, S. J., & Maskat, M. Y. (2017). Effects of deodorisation methods on volatile compounds, chemical properties and antioxidant activities of fucoidan isolated from brown seaweed (Sargassum sp.). *Algal Research, 25*, 507–515. https://doi.org/10.1016/j.algal.2017.06.018

Khan, F., Oh, D., Chandika, P., Jo, D.-M., Bamunarachchi, N. I., Jung, W.-K., & Kim, Y.-M. (2022). Inhibitory activities of phloroglucinol-chitosan nanoparticles on mono- and dual-species biofilms of *Candida albicans* and bacteria. *Colloids and Surfaces B: Biointerfaces, 211*, Article 112307. https://doi.org/10.1016/j.colsurfb.2021.112307

Koh, H. S. A., Lu, J., & Zhou, W. (2019). Structure characterization and antioxidant activity of fucoidan isolated from Undaria pinnatifida grown in New Zealand. *Carbohydrate Polymers, 212*, 178–185. https://doi.org/10.1016/j.carbpol.2019.02.040

Landa-Cansigno, C., Serviere-Zaragoza, E., Morales-Martínez, T. K., Ascacio-Valdes, J. A., Morreeuw, Z. P., Gauyat, C., … Reyes, A. G. (2023). The antioxidant and anti-elastase activity of the brown seaweed Sargassum horridum (Fucales, Phaeophyceae) and their early phenolics and saponins profiling for green cosmetic applications. *Algal Research, 75*, Article 103271. https://doi.org/10.1016/j.algal.2023.103271

Lee, Z. J., Xie, C., Duan, X., Ng, K., & Suleria, H. A. (2024). Optimization of ultrasonic extraction parameters for the recovery of phenolic compounds in Brown seaweed: Comparison with conventional techniques. *Antioxidants, 13*(4), 409.

Lee, Z. J., Xie, C., Ng, K., & Suleria, H. A. (2023). Unraveling the bioactive interplay: Seaweed polysaccharide, polyphenol and their gut modulation effect. *Critical Reviews in Food Science and Nutrition*, 1–24.

Li, B., Lu, F., Wei, X., & Zhao, R. (2008). Fucoidan: Structure and bioactivity. *Molecules, 13*(8), 1671–1695. https://doi.org/10.3390/molecules13081671

Li, H., Xu, S., Xie, Y., Zhang, Q., Ding, S., Wang, R., Fu, F., & Zhan, X. (2024). Curdlan-polyphenol complexes prepared by pH-driven effectively enhanced their physicochemical stability, antioxidant and prebiotic activities. *International Journal of Biological Macromolecules, 267*, Article 131579. https://doi.org/10.1016/j.ijbiomac.2024.131579

Liu, J., Lu, J.-F., Kan, J., Wen, X.-Y., & Jin, C.-H. (2014). Synthesis, characterization and in vitro anti-diabetic activity of catechin grafted inulin. *International Journal of Biological Macromolecules, 64*, 76–83. https://doi.org/10.1016/j.ijbiomac.2013.11.028

Liu, J., Wang, T., Huang, B., Zhuang, Y., Hu, Y., & Fei, P. (2021). Pectin modified with phenolic acids: Evaluation of their emulsification properties, antioxidation activities, and antibacterial activities. *International Journal of Biological Macromolecules, 174*, 485–493. https://doi.org/10.1016/j.ijbiomac.2021.01.190

Liu, Y., Ying, D., Sanguansri, L., Cai, Y., & Le, X. (2018). Adsorption of catechin onto cellulose and its mechanism study: Kinetic models, characterization and molecular simulation. *Food Research International, 112*, 225–232.

Ma, C., Zhou, X., Yu, H., Ni, J., Wang, X., Li, Y., & Wang, H. (2024). Virtual screening and multi-synergism of inhibitory compounds against pancreatic lipase. *Food Bioscience, 57*, Article 103601. https://doi.org/10.1016/j.fbio.2024.103601

Ma, Y., Chen, Z., Wang, Z., Chen, R., & Zhang, S. (2023). Molecular interactions between apigenin and starch with different amylose/amylopectin ratios revealed by X-ray diffraction, FT-IR and solid-state NMR. *Carbohydrate Polymers, 310*, Article 120737.

Manikandan, R., Parimalanandhini, D., Mahalakshmi, K., Beulaja, M., Arumugam, M., Janarthanan, S., … Prabhu, N. M. (2020). Studies on isolation, characterization of fucoidan from brown algae Turbinaria decurrens and evaluation of it's in vivo and in vitro anti-inflammatory activities. *International Journal of Biological Macromolecules, 160*, 1263–1276. https://doi.org/10.1016/j.ijbiomac.2020.05.152

Mercado-Mercado, G., de la Rosa, L. A., & Alvarez-Parrilla, E. (2020). Effect of pectin on the interactions among phenolic compounds determined by antioxidant capacity. *Journal of Molecular Structure, 1199*, Article 126967.

Mihai, E., Negreanu-Pirjol, B.-S., Craciunescu, O., Ciucan, T., Iosageanu, A., Seciu-Grama, A.-M., Prelipcean, A.-M., Utoiu, E., Coroiu, V., Ghenea, A.-M., & Negreanu-Pirjol, T. (2023). In vitro hypoglycemic potential, antioxidant and prebiotic activity after simulated digestion of combined blueberry pomace and chia seed extracts. *Processes, 11*(4), 1025. https://www.mdpi.com/2227-9717/11/4/1025.

Obluchinskaya, E. D., Pozharitskaya, O. N., & Shikov, A. N. (2022). In vitro anti-inflammatory activities of Fucoidans from five species of Brown seaweeds. *Marine Drugs, 20*(10), 606. https://www.mdpi.com/1660-3397/20/10/606.

Palanisamy, S., Vinosha, M., Marudhupandi, T., Rajasekar, P., & Prabhu, N. M. (2017). Isolation of fucoidan from *Sargassum polycystum* brown algae: Structural characterization, in vitro antioxidant and anticancer activity. *International Journal of Biological Macromolecules, 102*, 405–412. https://doi.org/10.1016/j.ijbiomac.2017.03.182

Peng, K., Li, Y., Sun, Y., Xu, W., Wang, H., Zhang, R., & Yi, Y. (2023). Lotus root polysaccharide-phenol complexes: Interaction, structure, antioxidant, and anti-inflammatory activities. *Foods, 12*(3), 577.

Rodriguez-Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., & Teixeira, J. A. (2011). Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers, 86*(3), 1137–1144. https://doi.org/10.1016/j.carbpol.2011.06.006

da Rosa, C. G., Borges, C. D., Zambiazi, R. C., Nunes, M. R., Benvenutti, E. V., Luz, S. R. D., … Rutz, J. K. (2013). Microencapsulation of gallic acid in chitosan, β-cyclodextrin and xanthan. *Industrial Crops and Products, 46*, 138–146. https://doi.org/10.1016/j.indcrop.2012.12.053

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., … Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in Pharmacology, 13*, Article 806470.

Sabando, C., Bouza, R., Rodríguez-Llamazares, S., Pettinelli, N., Ide, W., Barral, L., Linares, A., Quiroga, R., Castaño, J., Müller, N., & Dufresne, A. (2022). Donut-shaped microparticles prepared from different C-type starch sources: Characterization and encapsulation of Gallic acid. *ACS Food Science & Technology, 2*(5), 862–871. https://doi.org/10.1021/acsfoodscitech.2c00023

Sanniyasi, E., Gopal, R. K., Damodharan, R., Arumugam, A., Sampath Kumar, M., Senthilkumar, N., & Anbalagan, M. (2023). In vitro anticancer potential of laminarin and fucoidan from Brown seaweeds. *Scientific Reports, 13*(1), Article 14452. https://doi.org/10.1038/s41598-023-41327-7

Santoso, S. P., Bundjaja, V., Angkawijaya, A. E., Gunarto, C., Go, A. W., Yuliana, M., … Ju, Y.-H. (2021). One-step synthesis of nitrogen-grafted copper-gallic acid for enhanced methylene blue removal. *Scientific Reports, 11*(1), Article 12021. https://doi.org/10.1038/s41598-021-91484-w

Silva, F., Veiga, F., Cardoso, C., Dias, F., Cerqueira, F., Medeiros, R., & Paiva-Santos, A. C. (2024). A rapid and simplified DPPH assay for analysis of antioxidant interactions in binary combinations. *Microchemical Journal, 202*, Article 110801.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Subbiah, V., Ebrahimi, F., Duan, X., Agar, O. T., Barrow, C. J., & Suleria, H. A. R. (2024). Insights into the in vitro biological properties of Australian beach-cast brown seaweed phenolics. *Food Science & Nutrition*. https://doi.org/10.1002/fsn3.4415. n/a(n/a).

Taylor, K. A., & Buchanan-Smith, J. G. (1992). A colorimetric method for the quantitation of uronic acids and a specific assay for galacturonic acid. *Analytical Biochemistry, 201*(1), 190–196. https://doi.org/10.1016/0003-2697(92)90194-C

Tudorache, M., & Bordenave, N. (2019). Phenolic compounds mediate aggregation of water-soluble polysaccharides and change their rheological properties: Effect of different phenolic compounds. *Food Hydrocolloids, 97*, Article 105193. https://doi.org/10.1016/j.foodhyd.2019.105193

Wang, D., Mao, L., Dai, L., Yuan, F., & Gao, Y. (2018). Characterization of chitosan-ferulic acid conjugates and their application in the design of β-carotene bilayer emulsions with propylene glycol alginate. *Food Hydrocolloids, 80*, 281–291. https://doi.org/10.1016/j.foodhyd.2017.11.031

Wang, J., Yang, H., Luo, L., Ye, H., Xu, H., Sun, Y., Gong, L., & Yang, H. (2024). Persimmon leaf polyphenols as potential ingredients for modulating starch digestibility: Effect of starch-polyphenol interaction. *International Journal of Biological Macromolecules, 270*, Article 132524. https://doi.org/10.1016/j.ijbiomac.2024.132524

Wang, J., Zhang, Q., Zhang, Z., Song, H., & Li, P. (2010). Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from Laminaria japonica. *International Journal of Biological Macromolecules, 46*(1), 6–12. https://doi.org/10.1016/j.ijbiomac.2009.10.015

Wu, Z., Ming, J., Gao, R., Wang, Y., Liang, Q., Yu, H., & Zhao, G. (2011). Characterization and antioxidant activity of the complex of tea polyphenols and oat β-glucan. *Journal of Agricultural and Food Chemistry, 59*(19), 10737–10746. https://doi.org/10.1021/jf202722w

Xie, C., Leeming, M. G., Lee, Z. J., Yao, S., van de Meene, A., & Suleria, H. A. R. (2024). Physiochemical changes, metabolite discrepancies of brown seaweed-derived sulphated polysaccharides in the upper gastrointestinal tract and their effects on bioactive expression. *International Journal of Biological Macromolecules, 272*, Article 132845. https://doi.org/10.1016/j.ijbiomac.2024.132845

Xue, H., Du, X., Fang, S., Gao, H., Xie, K., Wang, Y., & Tan, J. (2024). The interaction of polyphenols-polysaccharides and their applications: A review. *International Journal of Biological Macromolecules, 134594*.

Yadav, P., Bandyopadhyay, A., Chakraborty, A., & Sarkar, K. (2018). Enhancement of anticancer activity and drug delivery of chitosan-curcumin nanoparticle via molecular docking and simulation analysis. *Carbohydrate Polymers, 182*, 188–198.

Yuan, Y., & Macquarrie, D. (2015). Microwave assisted extraction of sulfated polysaccharides (fucoidan) from *Ascophyllum nodosum* and its antioxidant activity. *Carbohydrate Polymers, 129*, 101–107. https://doi.org/10.1016/j.carbpol.2015.04.057

Zarandona, I., Puertas, A. I., Dueñas, M. T., Guerrero, P., & de la Caba, K. (2020). Assessment of active chitosan films incorporated with gallic acid. *Food Hydrocolloids, 101*, Article 105486. https://doi.org/10.1016/j.foodhyd.2019.105486

Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry, 44*(9), Article e13394.

Zhang, G., Zheng, C., Huang, B., & Fei, P. (2020). Preparation of acylated pectin with gallic acid through enzymatic method and their emulsifying properties, antioxidation activities and antibacterial activities. *International Journal of Biological Macromolecules, 165*, 198–204. https://doi.org/10.1016/j.ijbiomac.2020.09.195

Zhu, F. (2018). Interactions between cell wall polysaccharides and polyphenols. *Critical Reviews in Food Science and Nutrition, 58*(11), 1808–1883.