Thesis for the degree of Master of Engineering

Fractionation and structural characterization of antioxidant compounds from *Sargassum horneri* ethanol extract

Jaehong Shin

Department of Food Science and Engineering
The Graduate School
Jeju National University

February 2024

Fractionation and structural characterization of antioxidant compounds from *Sargassum horneri* ethanol extract

A Thesis submitted to the graduate school of
Jeju National University in partial fulfillment of
the requirements for the degree of Master of Engineering
under the supervision of Hyun Jung Kim

The thesis for the degree of Master of Engineering
by Jaehong Shin
has been approved by the dissertation committee.

2023. 12.

Chair Ji-Yeon Chun _____

Member Jongbin Lim _____

Member Hyun Jung Kim _____

CONTENTS

Fractionation and structural characterization of antioxidant compounds from *Sargassum horneri* ethanol extract

Jaehong Shin

Department of Food Science and Engineering
The Graduate School
Jeju National University

Abstract

Sargassum horneri (*S. horneri*), a brown seaweed, contains health-beneficial phytochemicals. Air pollutants, the particulate matter cause harmful effects on human health via reactive oxygen species generated by oxidative stress. In this study, we extracted *S. horneri* with ethanol (SHE) and SHE was further fractionated with n-hexane (S-SHE), chloroform (T-SHE), ethyl acetate (P-SHE), and water (PS-SHE). Also, bioactive compounds (sterols, simple sugars, and phenolic acids) in SHE, SHE-R (residue of SHE extraction), and SHE fractions were analyzed using high-performance liquid chromatography (HPLC) with DAD and RID. Furthermore, *in vitro* antioxidant activities, and antioxidant activities against particulate matter induced oxidative stress were investigated. The highest total phenolic contents were found in P-SHE (31.91 mg GAE/g) and flavonoid contents were in T-SHE (320.60 mg QE/g). The SHE-R contained the highest sulfate (14.93%) and T-SHE had the highest reducing sugar (0.06%). The predominant sterol detected in SHE, SHE-R, and SHE fractions was fucosterol, which ranged from 2.13 to 6.23 mg/100 g and other sterols were not found. Fucose, fructose, glucose, sucrose, maltose, and lactose were found in SHE and two to four kinds of sugars were

detected in SHE-R and SHE fractions. Gallic acid was the major phenolic acid compound in SHE and SHE fractions; however, no phenolic compound was detected in S-SHE. Either catechin or vanillic acid was found in T-SHE and P-SHE. The major phenolic compound in P-SHE was identified as gallic acid esterified glucose when analyzed using UPLC-Q-TOF-MS. SHE fractions showed higher antioxidant activities. Especially, P-SHE exhibited high activities in hydrogen peroxide scavenging (74.88%) and DPPH (70.63%), $ABTS^{+}$ (43.62%) and hydroxyl radical scavenging (27.65%). When the oxidative damage induced by particulate matter at 0.125 mg/mL, the antioxidant activities of SHE, SHE-R, and SHE fractions were mostly decreased. These results suggest that bioactive compounds in *S. horneri* can be isolated to be used as a natural source of antioxidants for functional food ingredients, and effectively suppress the oxidative stress induced by particulate matter.

LIST OF FIGURES

LIST OF TABLES

1. Introduction

Oxidative stress is caused by an oxidative imbalance between pro-oxidants and antioxidants when oxygen species are increased or antioxidants are decreased, resulting in the formation of toxic radicals. (Klran et al., 2023). Reactive oxygen species (ROS) include all unstable metabolites such as superoxide radical anion ($O2^{-}\cdot$), hydroxyl radical (·OH), and non-radicals, like singlet oxygen ($^{1}O_2$), and hydrogen peroxide (H_2O_2). These ROS are generated as a normal aerobic metabolism and could scavenge by the cellular endogenous antioxidant activities (Ozougwu, 2016). However, ROS are increased when stressed by excessive external environment, such as radiation, particulate matter, and chemical exposure (Rahal et al., 2014). In addition, the ROS are highly reactive and unstable, which could damage the cellular biomolecules such as proteins, lipids, and DNA (Gülçin, 2012). Their damage causes aging, cancer, diabetes, eye disease, atherosclerosis, cardiovascular diseases, rheumatoid arthritis, and kidney disease (Pisoschi et al., 2021; Waris and Ahsan, 2006).

Particulate matter (PM) is a serious pollutant of the urban atmosphere worldwide and, consists of extremely small particles containing polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Son et al., 2020). In general, PM has a particle size range of 10 to 50 μm, which is small enough to be easily inhaled by humans (Filonchyk M et al., 2016).

Components of PM negatively affect human health by enhancing ROS production, promoting inflammation, and inducing cell death (Tang et al., 2019). Because of the physicochemical properties, transition metals, and organic compounds of PM, PM-induced oxidative stress through ROS generation is a complex process (Libalova H et al., 2018). These properties could also affect NADPH-oxidases and a mitochondria function or expression

and generation of inflammatory cells (Møller et al., 2014.)

Antioxidative activity is the ability of a bioactive compound to maintain cellular structure and function by scavenging free radicals, inhibiting lipid peroxidation, and preventing other oxidative damage. (Zou et al., 2016). Antioxidants are used to neutralize and reduce ROS and prevent free radicals from damaging the body (Zulaikhah, 2017). In recent years, the use of synthetic antioxidants (e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG)) has been restricted due to the safety concerns (Michalak and Chojnacka, 2015; Xu et al., 2021). For this reason, there is a need to search for natural inhibitors of the oxidation process that do not have side effects.

Seaweeds are considered a rich source of antioxidants (phlorotannins, meroterpenoids, carotenoids, and pigments), with different types of antioxidants reported in different species (Bogolitsyn et al., 2019; Fernando et al., 2020; Osório et al., 2020; Sirbu et al., 2020). These seaweeds possess various bioactivities, such as anti-cancer, antibacterial, antifungal, and anti-inflammatory, which have the potential to be possible as medicinal and functional foods (Aminina et al., 2020; Gómez-Guzmán et al., 2018; Zhong et al., 2020). In particular, brown algae have been reported to have a relatively higher antioxidant activity than green and red algae (Dang et al., 2018). One of the significant polyphenols that determine the antioxidant activity of brown algae is the phlorotnannin, which polymers of phloroglucinol (1,3,5-tryhydroxybenzene) units, and have shown biological properties (Eom et al., 2012; Li et al., 2011). Phytosterols are essential components of the cell membranes and the most abundant in nature are β-sitosterol, campesterol, and stigmasterol. Among these, fucosterols are the predominant sterol in brown algae and have been reported to have health benefits (Abdul et al., 2016; Hikihara et al., 2020). Also, polysaccharides are one of the common and important bioactive compounds of brown algae that have the potential for

biological activities. The cell walls of brown algae contain sulfated polysaccharides such as fucoidan, laminarin, and alginic acid, which are absent in other algae (Dobrinčić et al., 2020). These sulfated polysaccharides consist of monosaccharides such as glucose, rhamnose, galactose, fucose, xylose, mannose, glucuronic acid, and mannuronic acid. The major functions of seaweed polysaccharides are antioxidant, antitumor, anti-inflammatory, and antiviral (Costa et al., 2010; Vishchuk et al., 2011).

Sargassum horneri (*S. horneri*) is an edible brown algae used as a functional ingredient in traditional medicine in Asian countries (Herath et al., 2020). Nowadays, a considerable amount of *S. horneri* is moving by ocean currents from the east coast of China to the coast of Korea, particularly on Jeju Island. As a result, a large amount of strains are present on the coast of Jeju Island (Kim et al., 2018). Although *S. horneri* has been subjected to research bioactive compounds and their biological activities (Kim et al., 2022; Saraswati et al., 2019; Shao et al., 2014), their antioxidant activities of fractions obtained by different solvent types have not been studied in much detail.

The objectives of this study were (1) to prepare different solvent fractions of *S. horneri* ethanol extract (SHE), (2) to compare antioxidant properties of SHE fractions, (3) to analyze bioactive compounds of SHE fractions, and (4) to evaluate the efficacy of SHE fractions on PM-induced oxidative damage.

2. Materials and methods

2.1. Materials

n-Hexane, ethanol, and ethyl acetate from J. T. Baker (Philipsburg, NJ, USA). Chloroform, Folin & Ciocalteu's phenol reagent, gelatin from bovine skin, Type B, barium chloride, aluminum chloride, 3,5-dinitrosalicylic acid (DNS), 2,2-diphenyl-1-picrythydrazyl (DPPH), formic acid, potassium persulfate, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), hydrogen peroxide solution, potassium phosphate monobasic, potassium phosphate dibasic, peroxidase from horseradish, ferric chloride, iron (II) sulfate heptahydrate, 2-deoxy-D-ribose, iron (II) chloride, potassium ferricyanide (III), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Campesterol, stigmasterol, β-sitosterol, fructose, glucose, sucrose, maltose, polyphenol standards including gallic acid, protocatechuic acid, chlorogenic acid, quercetin, myricetin, trans-ferulic acid, p-coumaric acid, vanillic acid, catechin, gentisic acid, and caffeic acid were purchased from Sigma-Aldrich as well. Acetic acid, fucose, and lactose were purchased from TCI Chemicals (Tokyo, Japan). Fucosterol was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Methanol, acetonitrile, and water from Burdick & Jackson Co. (Muskegon, MI, USA). Sodium carbonate, sodium nitrite, sodium hydroxide, hydrochloric acid, potassium sulfate, sulfuric acid, potassium sodium tartrate, and trichloroacetic acid (TCA) were obtained from Daejung Chemical Co. (Siheung, Korea). All chemicals used in the current study were of analytical or high-performance liquid chromatography (HPLC) grade.

2.2. Particulate matter (PM)

Certified Reference Material (CRM No. 28) for urban aerosols collected over 10 years in Beijing, China was purchased from the National Institute for Environmental Studies (NIES, Ibaraki, Japan). The diameters of fine particles in PM are mainly less than 10 μm. The metal elemental compositions of PM are certified as Na, Mg, Al, K, Ca, Ti, Fe, and Zn, respectively. The particle diameters and concentrations of PM have been previously reported (Mori et al., 2008). The PM was suspended in RPMI and stored at －80℃.

2.3. Extraction and fractionation of *Sargassum horneri*

The extraction and fractionation process of *Sargassum horneri* (*S. horneri*) are shown in Fig. 1.

S. horneri was collected from the coast of Jeju, Korea. The collected *S. honeri* was washed thrice with pure water and subjected to hot-air drying at 50°C. The air-dried *S. horneri* was ground and passed through as 40-50 mesh by pin-mill. The *S. horneri* powder was extracted 70% ethanol for 24 h and filtered with white-clay for 2 h to remove residues and metals. After filtration, the extracts were centrifuged at 1,200 rpm at room temperature. Finally, the supernatant was concentrated and treated with 95% ethanol to acquire pure extracts. The purified *S. horneri* extracts were concentrated to 20% of the solid content and were freeze-dried. The ethanol extract (SHE) and residue of SHE (SHE-R) were stored at －20°C until use.

The SHE was dissolved in distilled water and partitioned with a separatory funnel using three different solvents as shown in Fig. 1., namely, n-hexane (S-SHE), chloroform (T-SHE), ethyl acetate (P-SHE), and aqueous fraction (PS-SHE) to fractionate polar and non-polar compounds. The solvent fractions were concentrated by a rotary evaporator (SB-1200; Shanghai

EYELA Co., Shanghai, China). The resulting fractions were stored at −20°C until use.

2.4. Determination of total polyphenol and total flavonoid contents

The total phenolic content was determined by the Folin–Ciocalteu method of Wang et al. (2011). The sample (100 μL) was mixed with 1.5 mL of distilled water and 100 μL of 2 N Folin–Ciocalteu reagent. Then, 300 μL of 20% sodium carbonate was added and the mixtures were placed at room temperature for 1 h in the dark. The absorbance was measured at 765 nm using a UV–vis spectrophotometer (OPTIZEN 2120UV; Mecasys, Daejeon, Korea). The total polyphenol content was shown as gallic acid equivalents (mg GAE/g of sample).

The total flavonoid content was measured by the method of Yi et al. (2017). Each sample (200 μL) was mixed with 800 μL of ethanol and 60 μL of 5% $NaNO_2$, and incubated at room temperature. After 5 min, the mixture was reacted with 60 μL of 10% $AlCl_3$ and allowed to stand for 5 min. Subsequently, 400 μL of 1 M NaOH and 500 μL of distilled water were added to mixture. The absorbance was measured at 415 nm UV–vis spectrophotometer (Mecasys). The total flavonoid content was expressed as quercetin equivalent (mg QE/g of sample).

2.5. Determination of sulfate group content

The sulfate content was measured using the barium chloride–gelatin method of Ruiz et al. (2007) and Mohd Fauziee et al. (2021). The sample (1 mg) was hydrolyzed using 1 M HCl (1 mL) at 100°C for 6 h. After hydrolysis, the mixtures were cooled to room temperature. An aliquot (0.2 mL) of the hydrolysate was transferred into test tubes, where two sets of test tubes

were prepared for each sample (Set A and Set B). Set A, containing 3.8 mL of 3% (w/v) trichloroacetic acid and 1 mL of $BaCl_2$-gelatin reagent. Meanwhile, Set B contains 3.8 mL of 3% (w/v) trichloroacetic acid and 1 mL of gelation reagent (0.5% w/v). The samples were mixed for 20 min and measured corresponding reagent blanks (Set A and Set B without samples). A set of standards of potassium sulfate was prepared and measured against Set A reagent blank. The absorbance of the sample was measured at 360 nm using a UV - vis spectrophotometer (Mecasys).

2.6. Determination of reducing sugar

For determining reducing sugar contents of SHE fractions, polysaccharides present in SHE were hydrolyzed to form reduced sugars using H_2SO_4. 100 mg of samples were added to 10 mL of 1.5 M H_2SO_4 in a test tube. Hydrolysis was achieved by heating this mixture to 100℃ by keeping it in boiling water for 20 min. The hydrolyzed mixture was neutralized with 12 mL of 10% NaOH. Then, the obtained solution was transferred into a 100-mL volumetric flask, and the volume was made up of distilled water. The reducing sugars were determined by the dinitrosalicylic acid (DNS) method, with slight modification to the method of Kim and Yi (2010). 1 mL of hydrolyzed sample mixture was added with 2 mL of distilled water and 1 mL of DNS solution in a test tube. Test tubes were capped and heated to 100℃ by keeping them in boiling water for 5 min. Samples were cooled to room temperature under running tap water. The absorbance of the sample was measured at 540 nm using a UV - vis spectrophotometer (Mecasys). The reducing sugar was calculated using a standard curve with glucose solutions of different concentrations.

2.7. Method validations for HPLC analysis

Method validation of HPLC analysis was performed according to the single laboratory method validation procedure of AOAC (2002). The linearity of the sterols, monosaccharides and disaccharides, and phenolic acids analysis was tested at five different concentrations of sterols (fucosterol, campesterol, β-sitosterol, and stigmasterol) and monosaccharides and disaccharides (fucose, fructose, glucose, sucrose, maltose, and lactose), and six different concentrations of phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid, quercetin, myricetin, trans-ferulic acid, p-coumaric acid, vanillic acid, catechin, gentisic acid, and caffeic acid) standard solutions. All analyses were performed in triplicate. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using calibration curve. The LOD was calculated based on the detector signal-to-noise (S/N) ratio by multiplying the standard deviation of the S/N ratio by 3.3 and adding this to the average of the S/N ratio. The LOQ was obtained by using 10 as the multiplying factor. The LOD and LOQ values are presented as μg/injection volume.

2.8. Analysis of sterols, simple sugars, and phenolic acids by HPLC

Sterols of SHE and its fractions were analyzed by HPLC (Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA) with a diode array detector (G7115A DAD WR, Agilent Technologies) and a poroshell 120 EC-C18 column (250 x 4.6 mm, 4 μm, Agilent Technologies) (Lopes et al., 2011). The mobile phase was 70% acetonitrile at a flow rate of 1.2 mL/min. The injection volume was 20 μL while the column temperature was maintained at 30℃, and the detector was set at 205 nm. Sterols in the samples were identified by comparing their retention times with the corresponding data obtained by analyzing the standard compounds. Sterol

concentrations in the samples were calculated as the average peak area after duplicate injections and expressed in mg/100 g.

Monosaccharides and disacharrides as simple sugars were analyzed by HPLC with a refractive index detector (G7162A RID, Agilent Technologies) and a ZORBAX NH2 column (150 x 4.6 mm, 5 μm, Agilent Technologies). The mobile phase was 80% acetonitrile at a flow rate of 1.0 mL/min. The injection volume was 10 μL while the column and detector temperature were maintained at 35℃. Monosaccharides and disaccharides were identified by comparison of the retention time in chromatogram with standard compounds. Concentration in the samples was calculated using the average peak and expressed in mg/100 g.

Phenolic acids were analyzed by HPLC-DAD and a reversed-phase Pursuit XRs C18 column (250 x 4.6 mm, 5 μm, Agilent Technologies). The composition of solvents and used gradient elution conditions were previously described (López et al., 2011). Mobile phases consisted of 0.1% formic acid in water (eluent A) and acetonitrile (eluent B) at a flow rate of 1.0 mL/min. The elution conditions were as follows: 0-1 min, 100-80% A; 1-8 min, 80-40% A; 8-10 min, 40-80%; 10-12 min, 80% A. The injection volume was 20 μL while the column temperature was maintained at 27℃. The detector was set at 270 nm (gallic acid, protocatechuic acid, catechin, and vanillic acid), 324 nm (chlorogenic acid, gentisic acid, caffeic acid, coumaric acid, and ferulic acid), and 373 nm (myricetin and quercetin). Polyphenol compounds were identified by comparison of the retention time in chromatogram with standard. The polyphenol concentrations in the samples were calculated as the average peak area and expressed in mg/100 g.

2.9. Identification of phenolic compounds using ultra performance liquid chromatography-quadrupole-time-of-flight/mass spectrometry (UPLC-Q-TOF-MS)

The masses of phenolic compounds were determined using UPLC-Q-TOF-MS (Xevo G2-XS QTof, Waters, Milford, MA, USA). Briefly, 1.0 μL of the P-SHE in ethyl acetate was injected onto an Acquity UPLC BEH C18 column (2.1 mm × 100 mm; 1.7 μm; Waters) operating at 40℃ with a flow rate of 0.35 mL/min. The mobile phase was HPLC water (A) and acidified acetonitrile (0.1% formic acid) (B). Compounds were separated using the following gradient conditions: 0 min, 1% B isocratic; 1 min, 1 % B isocratic; 1-8 min linear gradient from 100% B; 8-9 min, 100% B isocratic; 9.5 min, 1% B isocratic; 9.5-12 min, 1% B, followed by washing and reconditioning of the column. Optical conditions for the electrospray interface were as follows: desolvation gas flow 1,000 L/h, cone gas flow 10 L/h, desolvation temperature 500℃, ion source temperature 80℃, capillary voltage 2.5 kV, and sample cone voltage 40 V. The MS system was operated in negative ion mode with the mass range set at m/z 50-1500 with a scan time of 0.2 sec using Waters Masslynx software (v 4.1). Peak identification was performed using UNIFI (1.8, Waters Corp., Milford, MA, USA).

2.10. *In* vitro antioxidant activity

2.10.1. DPPH radical scavenging activity

The DPPH (1,1-diphenyl-2-pucrylhydrazyl) radical scavenging activity was measured by the method of Kim et al. (2006). Each sample (70 μL) was mixed with 140 μL of DPPH solution (1 mM in ethanol). The mixtures were incubated at room temperature for 30 min. The absorbance was measured at 517 nm using a microplate reader (Epoch™; BioTek Instruments Inc., Winooski, VT, USA). The activity was expressed as a percentage of the DPPH and was calculated using the following equation:

DPPH scavenging activity (%) = [1-(A_{sample}/A_{blank})] x 100.

2.10.2. ABTS^{+} radical scavenging ability

The ABTS^{+} radical scavenging activity was determined according to the method of Sung et al. (2018). The ABTS^{+} radical solution was prepared by 7 mM ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) aqueous solution with 2.45 mM potassium persulfate aqueous solution in equal quantities and allowed them to react for 16 h. Then, 20 μL of the sample was mixed with 180 μL of ABTS^{+} radical solution. The absorbance was measured at 734 nm using a microplate reader (BioTek Instruments Inc.).

2.10.3. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured by the method of Heo et al. (2005). Each sample (100 μL) and 20 μL of 10 mM hydrogen peroxide were mixed with 100 μL of 0.1 M phosphate buffer (pH 7.4) in a 96-well plate and incubated at 37℃ for 5 min. Subsequently, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit/mL) were added to the mixture and incubated at 37℃ for 10 min. The absorbance at 405 nm was measured using a microplate reader (BioTek Instruments Inc.). The hydrogen peroxide radical scavenging activity was calculated following equation:

Hydrogen peroxide scavenging activity (%) = [(1-(A_{sample}-$A_{smaple\ control}$))/A_{blank}] x 100,

where A_{sample} is the absorbance of the sample, $A_{sample\ control}$ is the absorbance of the sample itself, and A_{blank} is the absorbance of distilled water.

2.10.4. Hydroxyl radical scavenging activity

Hydrogen peroxide scavenging activity was measured by the method of Heo et al. (2005). Each sample (100 μL) and 20 μL of 10 mM hydrogen peroxide were mixed with 100 μL of 0.1 M phosphate buffer (pH 7.4) in a 96-well plate and incubated at 37℃ for 5 min. Subsequently, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit/mL) were added to the mixture and incubated at 37℃ for 10 min. The absorbance at 405 nm was measured using a microplate reader (BioTek Instruments Inc.). The hydrogen peroxide radical scavenging activity was calculated following equation:

Hydrogen peroxide scavenging activity (%) = $[(1-(A_{sample}-A_{smaple\ control}))/A_{blank}]$ x 100,

where A_{sample} is the absorbance of the sample, $A_{sample\ control}$ is the absorbance of the sample itself, and A_{blank} is the absorbance of distilled water.

2.10.5. Ferrous ion chelating effect

The ferrous ion chelating effect was measured according to the method of Lee et al. (2010). Each sample (100 μL) was mixed with 100 μL of $FeCl_2$ (0.1 mM). Then, 100 μL of 0.25 mM ferrozine was added and incubated at room temperature for 10 min in the dark. After the reaction, the absorbance was determined at 562 nm using a microplate reader (BioTek Instruments Inc.). The metal ion chelating effect was determined as follows:

Ferrous ion chelating effect (%) = $[1-(A_{sample}/A_{blank})]$ x 100.

2.10.6. Reducing power

The reducing power was determined by the method of Sabeena Farvin and Jacobsen (2013). Each sample (1 mL) was mixed with 0.2 M phosphate buffer (pH 6.6, 1 mL) and 1% potassium ferricyanide (1 mL), and incubated at 50℃ for 20 min. Subsequently, 1 mL of 10% TCA was added and an aliquot of

the mixture (2 mL) was incubated with 2 mL of distilled water and 400 μL of 0.1% ferric chloride at room temperature for 10 min. The absorbance was measured at 700 nm using a UV - vis spectrophotometer (Mecasys). Ascorbic acid was used as a positive control.

2.11. Statistical analysis

Statistical analyses were performed using SPSS Statistics (IBM Co., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance followed by Duncan's multiple range test. The $p<0.05$ was considered as statistically significant. The correlation was expressed as Pearson's correlation coefficients.

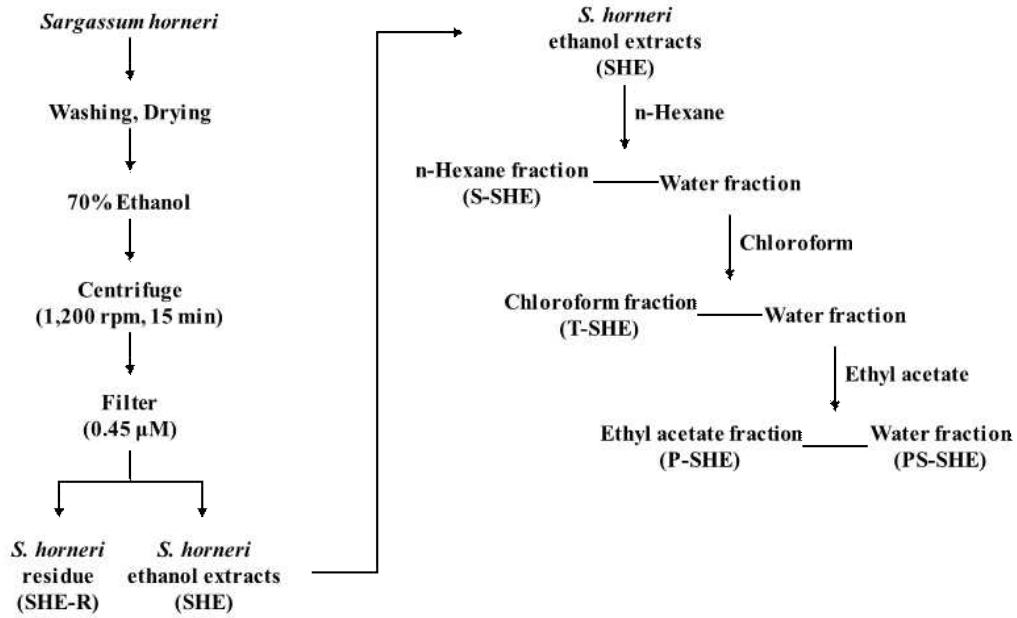

Fig. 1. Preparation of *Sargassum horneri* extract and four different solvent fractions

3. Results and Discussion

3.1. Yields of SHE solvent-partitioned fractions

Because a single solvent is not sufficient to extract phytochemicals, solvents with different polarities are used to extract phytochemicals and antioxidant compounds based on their chemical properties (Nawaz et al., 2020). The yields of the fractions partitioned by n-hexane, chloroform, ethyl acetate, and water from SHE are shown in Table 1. Among the solvent-partitioned fractions, S-SHE from n-hexane fraction of SHE showed the highest extraction yield (75.35%), followed by T-SHE (13.41%), P-SHE (2.46%), and PS-SHE (1.07%) fractions. These results show that there were significant differences in the composition and ratio of SHE, which were partitioned by polarities. The extraction yield of chemical extraction process is dependent on various factors such as solvent polarity, extraction time, temperature, and chemical composition of the sample (Kaneria et al., 2012). In particular, there have been many reports of variations in extractive yield with different solvents. In green seaweeds, n-hexane fraction (43.6%) from *E. prolifera* showed the highest yield followed by chloroform fraction (25.9%), aqueous fraction (20.2%), and ethyl acetate fraction (5.5%) (Cho et al., 2011). In other types of brown seaweed, *E. cava* Kjellman, showed the yields of the n-hexane fraction (33.42%), ethyl acetate fraction (9.71%), n-butanol fraction (14.48%), and aqueous fraction (42.39%) (Cho et al., 2012).

Phytochemical compounds of plants have different polarity so that they can be extracted by different polarity of solvent (Dehkharghanian et al., 2010). n-Hexane could dissolve non-polar compounds, such as lipids, lignins, aglycons, and sterols, whereas water is effective in extracting sugars, amino acids, and glycoside compounds (Widyawati et al., 2014). Franco et al. (2008)

reported that ethyl acetate has been widely used to extract polyphenols from plants, and chloroform can extract terpenoids and flavonoids (Yin et al., 2013). In the current study, the fraction partitioned by n-hexane was called as S-SHE, sterol-rich compound containing SHE. Like this, the chloroform fraction was T-SHE, terpenoid-rich SHE, the ethyl acetate fraction was P-SHE, polyphenol-rich SHE, and the water fraction was PS-SHE, polysaccharide-rich SHE.

Table 1. Extraction yields of four different fractions from *S. horneri* ethanol extract (SHE)

Fractions	Extraction yield (%)
S-SHE[1)]	75.35±8.79
T-SHE	13.41±3.82
P-SHE	2.46±1.96
PS-SHE	1.07±0.35

1) S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

3.2. Total phenolic and flavonoid contents

The total phenolic (TPC) and flavonoid contents (TFC) of SHE extract and its solvent fractions are shown in Table 2. The TPC of the SHE and SHE-R were 16.57 and 17.53 mg GAE/mg, which has no significant differences ($p>0.05$). On the other hand, the TFC of SHE and SHE-R were 58.61 and 24.00 mg QE/g, respectively. The SHE showed higher TFC than the SHE-R. Among four different solvent fractions, the P-SHE showed the highest amount of TPC (31.91 mg GAE/g), followed by T-SHE (24.18 mg GAE/g), PS-SHE (18.37 mg GAE/g), and S-SHE (16.76 mg GAE/g). The TPC of P-SHE and T-SHE were significantly higher than that of S-SHE and PS-SHE. The highest TFC in four solvent fractions was that of T-SHE at 320.60 mg QE/g, followed by P-SHE (303.77 mg QE/g), S-SHE (92.38 mg QE/g), and PS-SHE (38.38 mg QE/g), which showed a similar tendency to TPC. Therefore, two fractions, T-SHE (terpenoid-rich) and P-SHE (polyphenol-rich fraction) had the higher amounts of both TPC and TFC than other two fractions, S-SHE (sterol-rich fraction) and PS-SHE (polysaccharide-rich fraction).

3.3. Sulfate group contents and reducing sugars

The sulfate group contents of *S. horneri* extract and four different solvent fractions ranged from 6.43 to 14.83% (Table 2). The high sulfate group contents were found in SHE-R (12.43%) and PS-SHE (12.53%). SHE-R is the residue after the extraction of SHE, which might contain high amounts of carbohydrates. And PS-SHE is partitioned by water and rich in polysaccharides. Mostly, in seaweeds, polysaccharides are present with sulfate groups (García-Ríos et al., 2012). High content of sulfate group in polysaccharides is related to the beneficial biological activity (Bak et al., 2021), therefore the quantification of sulfate groups is significant.

Reducing sugars were only found in SHE-R and T-SHE, which were 0.01 and 0.06% respectively (Table 2). The low reducing sugar content found in SHE extracts and fractions was due to insufficient hydrolysis. According to Meillisa et al. (2015), when *S. japonica* was hydrolyzed using subcritical water and formic acid as a catalyst, the reducing sugar content was found to be 0.04 to 0.05 g/L. In other brown seaweeds, *U. prolifera* showed the reducing sugars ranged from 0.037 to 0.152 g/gdw, for hydrolyzed with sulfuric acid 121℃, 15 psi using an autoclave (Dave et al., 2021). Offei et al. (2018) reported that brown algae, *F. serratus*, was hydrolyzed with 0.5 M sulfuric acid at 121℃ for 15 min, the reducing sugar content was 305 mg/g dry matter, where *U. pinnatifida* was hydrolyzed with 0.075 M at 121℃ for 60 min, the reducing sugar content was 220 mg/g dry matter. The hydrolysis factor depends on the sample concentration, time, acid concentration, and temperature (Hessami et al., 2019). Especially, in brown algae extraction, reducing sugar content generally increases with extraction time (Park et al., 2008).

Table 2. Total polyphenol, flavonoid, and sulfate group contents, and reducing sugar of *S. horneri* extract and its solvent fractions

	Total polyphenol contents (mg GAE/g)	Total flavonoid contents (mg QE/g)	Sulfate group contents (%)	Reducing sugar (%)
SHE[1)]	17.53±0.71^{c}[2)]	58.61±4.10^{d}	10.73±0.85^{bc}	–[3)]
SHE-R	16.57±0.15^{c}	24.00±2.91^{f}	14.83±0.85^{a}	0.01±0.00^{b}
S-SHE	16.76±0.73^{c}	92.38±4.62^{c}	6.47±2.71^{d}	–
T-SHE	24.18±2.52^{b}	320.60±13.68^{a}	9.33±0.85^{cd}	0.06±0.00^{a}
P-SHE	31.91±2.06^{a}	303.77±7.08^{b}	6.43±2.37^{d}	–
PS-SHE	18.37±1.10^{c}	38.38±2.65^{e}	12.53±0.76^{ab}	–

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) Each value is mean±standard deviation. Means with different small letters in the same column are significantly different ($p < 0.05$).

3) – means not detected.

3.4. Method validation of HPLC analysis

The HPLC methods for the analysis of sterols, simple sugars, and phenolic acids were validated by the determination of the linearity, limit of detection (LOD), and limit of quantitation (LOQ). The regression data for the calibration curves and the LOD and LOQ calculations of the peaks are shown in Table 3.

The linearity of sterols analysis, expressed by the linear correlation coefficients (R^2) of fucosterol, campesterol, β-sitosterol, and stigmasterol were 0.9997, 0.9990, 0.9995, and 0.9962, respectively. The LOD and LOQ of HPLC analysis of fucosterol were 0.04 and 0.13 μg/injection volume (20 μL), campesterol (LOD; 0.01, LOQ; 0.04), β-sitosterol (LOD; 0.01, LOQ; 0.04), and stigmasterol (LOD; 0.03, LOQ; 0.09).

For simple sugars, the correlation coefficients of fucose, fructose, glucose, sucrose, maltose, and lactose were 0.9994, 0.9908, 0.9996, 0.9997, 0.9989, and 0.9985, respectively. The LOD and LOQ values for fucose were 0.04 and 0.12 μg/injection volume (10 μL), followed by 0.14 and 0.42 for fructose, 0.01 and 0.03 for glucose, 0.00 and 0.01 for sucrose, 0.05 and 0.15 for maltose, and 0.05 and 0.15 μg/injection volume for lactose.

For phenolic acids analysis, eleven phenolic acid compounds showed excellent correlation coefficients, which were greater than or equal to 0.99 for all calibration curves. The LOD value was 0.04 to 1.85 μg/injection volume (20 μL), and the LOQ value was 0.12 to 5.60 μg/injection volume for each phenolic acid.

These results indicate that the method validation exhibited good linearity (R^2<0.99) and supports the lack of bias in results for these analytes run in separate analytical batches.

Table 3. Method validation data for the quantitative determination of sterols, sugars, and phenolic acids by HPLC analysis

Compound		Calibration curve equation (y=Ax+B)	Correlation of coefficient (R^2)	LOD[1)]	LOQ[2)]
Sterols	Fucosterol	y=0.0003x-0.0163	0.9997	0.04	0.13
	Campesterol	y=0.0001x+0.0057	0.9990	0.01	0.04
	β-Sitosterol	y=0.0001x-0.0181	0.9995	0.01	0.04
	Stigmasterol	y=0.0001x-0.0557	0.9962	0.03	0.09
Sugars	Fucose	y=0.000002x-0.0043	0.9994	0.04	0.12
	Fructose	y=0.00001x+0.0141	0.9908	0.14	0.42
	Glucose	y=0.000002x-0.008	0.9996	0.01	0.03
	Sucrose	y=0.000002x-0.0119	0.9997	0.00	0.01
	Maltose	y=0.000002x-0.023	0.9989	0.05	0.15
	Lactose	y=0.000002x+0.0327	0.9985	0.08	0.23
Phenolic acids	Gallic acid	y=0.0164x-1.8925	0.9990	0.58	1.75
	Protocatechuic acid	y=0.0194x+0.1811	0.9997	0.18	0.53
	Catechin	y=0.136x+0.8751	0.9996	0.38	1.15
	Vanillic acid	y=0.0186x+0.2502	0.9999	0.24	0.72
	Chlorogenic acid	y=0.0155x-3.6115	0.9906	0.07	0.20
	Caffeic acid	y=0.0098x+0.9516	0.9999	0.30	0.92
	Gentisic acid	y=0.0272x+0.2625	0.9999	0.28	0.85
	p-Coumaric acid	y=0.0093x+0.153	0.9999	0.04	0.12
	trans-Ferulic acid	y=0.0096x+0.1421	1.0000	0.04	0.12
	Myricetin	y=0.015x-0.0142	1.0000	1.85	5.60
	Quercetin	y=0.0145x+0.9365	0.9994	0.37	1.12

[1)] Limit of detection (μg/injection volume).

[2)] Limit of quantitation (μg/injection volume).

3.5. Sterol compounds

Sterol compounds such as fucosterol, campesterol, β-sitosterol, and stigmasterol were determined in *S. horneri* extracts and fractions of SHE and are shown in Fig. 2. and Table 4. Among four sterol compounds, only fucosterol was detected in SHE and its fractions. The highest fucosterol was detected in S-SHE at 25.96 mg/100 g, followed by SHE (6.23 mg/100 g), T-SHE (3.72 mg/100 g), PS-SHE (2.40 mg/100 g), P-SHE (2.28 mg/100 g), and SHE-R (2.13 mg/100 g).

There are several sterol compounds present in seaweeds. Fucosterol is found in brown algae, cholesterol in red algae, and β-sitosterol in green algae (Hannan et al., 2020). Patterson (1971) reported that the major sterol in brown algae was fucosterol and it has many biological activities, such as anti-cancer, anti-diabetic, and cholesterol-reducing properties (Chakraborty et al., 2015). In the present study, fucosterol was also the major sterol in *S. horneri* extracts and their solvent fractions. Early study reported that the HPLC analysis of brown seaweeds, *H. elongate*, *U. pinnatifid*, and *L. ochroleuc* contained higher sterol contents than red seaweeds did, which ranged from 662 to 2320 μg/g (Sánchez-Machado et al., 2004). Among four different fractions, S-SHE contained the highest fucosterol, therefore S-SHE is called a sterol-rich fraction. n-Hexane is a non-polar solvent which intended to extract non-polar sterols. According to Milović et al. (2019), fucosterol was the predominant sterol in brown seaweed, *C. barbata*. Also, it was noted that fucosterol is a typical sterol for brown algae (Cvitković et al., 2021).

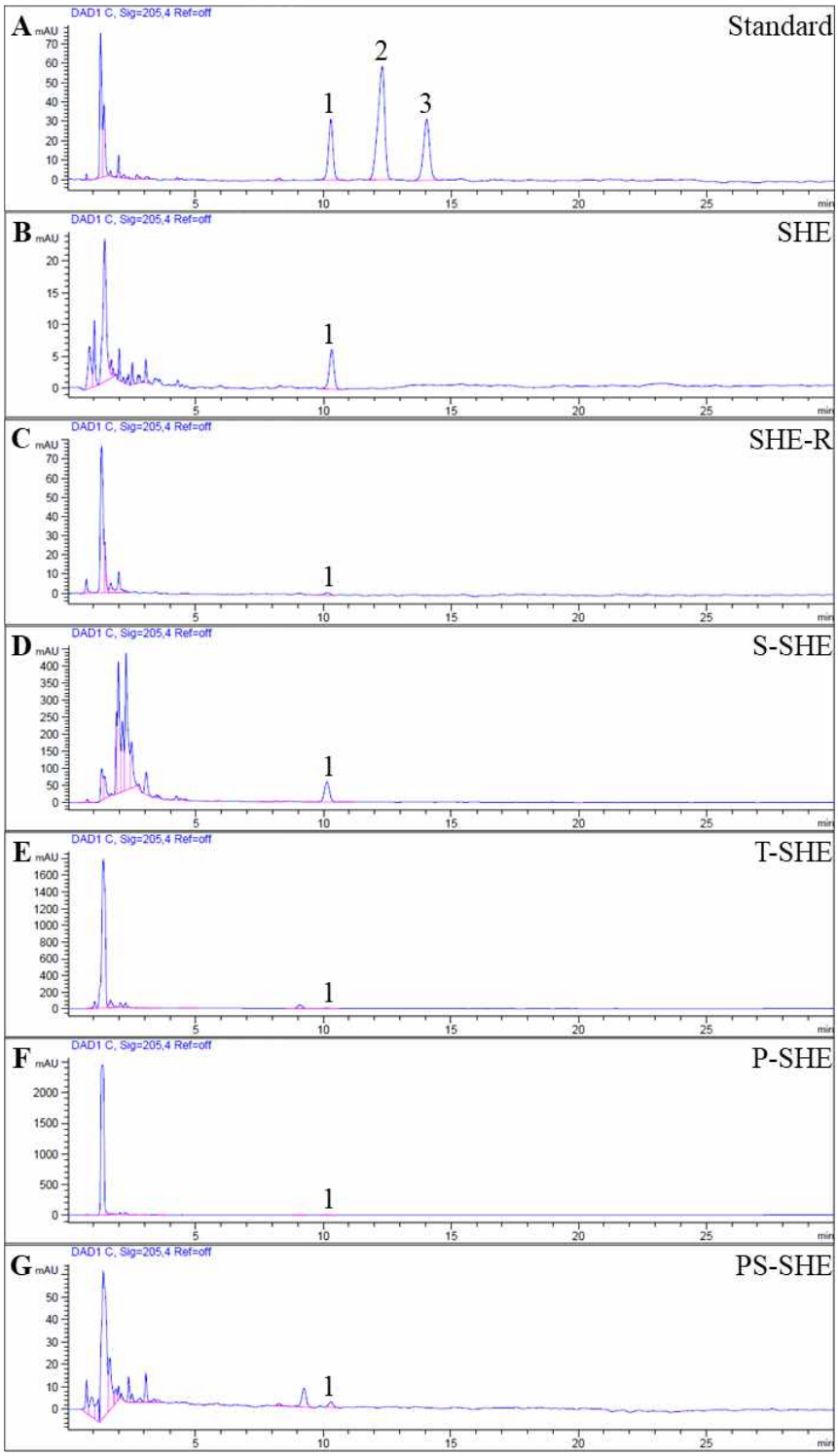

Fig. 2. HPLC-DAD chromatograms of (A) standard solution of sterols, (B) SHE, (C) SHE-R, (D) S-SHE, (E) T-SHE, (F) P-SHE, and (G) PS-SHE. Peaks: fucosterol (1), stigmasterol + campesterol (2), and β-sitosterol (3).

Table 4. Sterol contents in *S. horneri* extract and its solvent fractions

Sterol	*S. horneri* extract and fractions (mg/100 g)					
	SHE[1)]	SHE-R	S-SHE	T-SHE	P-SHE	PS-SHE
Fucosterol	6.23±0.14^{b}[2)]	2.13±0.14^{d}	25.96±0.47^{a}	3.72±0.12^{c}	2.28±0.22^{d}	2.40±0.14^{d}
Campesterol	–[3)]	–	–	–	–	–
β-Sitosterol	–	–	–	–	–	–
Stigmasterol	–	–	–	–	–	–

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) Each value is mean±standard deviation. Means with different small letters in the same row are significantly different ($p < 0.05$).

3) – means not detected.

3.6. Sugar compounds

Monosaccharides and disaccharides, such as fucose, fructose, glucose, sucrose, maltose, and lactose were determined in *S. horneri* extracts and fractions of SHE and are shown in Fig. 3. and Table 5.

Six different kinds of sugars were detected in SHE with lactose being the most abundant at 3.78 mg/100 g. In SHE-R, only sucrose and lactose were detected, as 1.77 and 3.70 mg/100 g respectively. Fucose was detected in all SHE fractions, with T-SHE containing the highest (1.34 mg/100 g), followed by PS-SHE (1.20 mg/100 g), P-SHE (0.92 mg/100 g), and S-SHE (0.65 mg/100 g). In addition, fructose was detected in all fractions except T-SHE, which ranges from 1.50 to 1.57 mg/100 g. Glucose was detected only in PS-SHE at 1.04 mg/100 g, whereas maltose was detected at 0.76 and 0.54 mg/100 g in S-SHE and T-SHE. Lactose was only detected in S-SHE (3.73 mg/100 g). According to the results of sugar compounds, the predominant monosaccharides and disaccharides present in *S. horneri* extracts and its fractions was fucose, which is a deoxy-galactose, a sub-unit of polysaccharide fucoidans. Previous study reported that polysaccharides in brown seaweeds (*S. japonica*, *U. pinnatifida*, *S. fusiforme*, and *S. hemiphyllum*) were mainly composed of glucose, fucose, galactose, and mannose (Chen et al., 2021). Further, Zhang et al. (2022) also reported major monosaccharides in brown algae were glucose and fucose, while red algae are rich in galactose and xylose. These results suggested polysaccharides from different species of brown seaweed exhibited different composition of sugars.

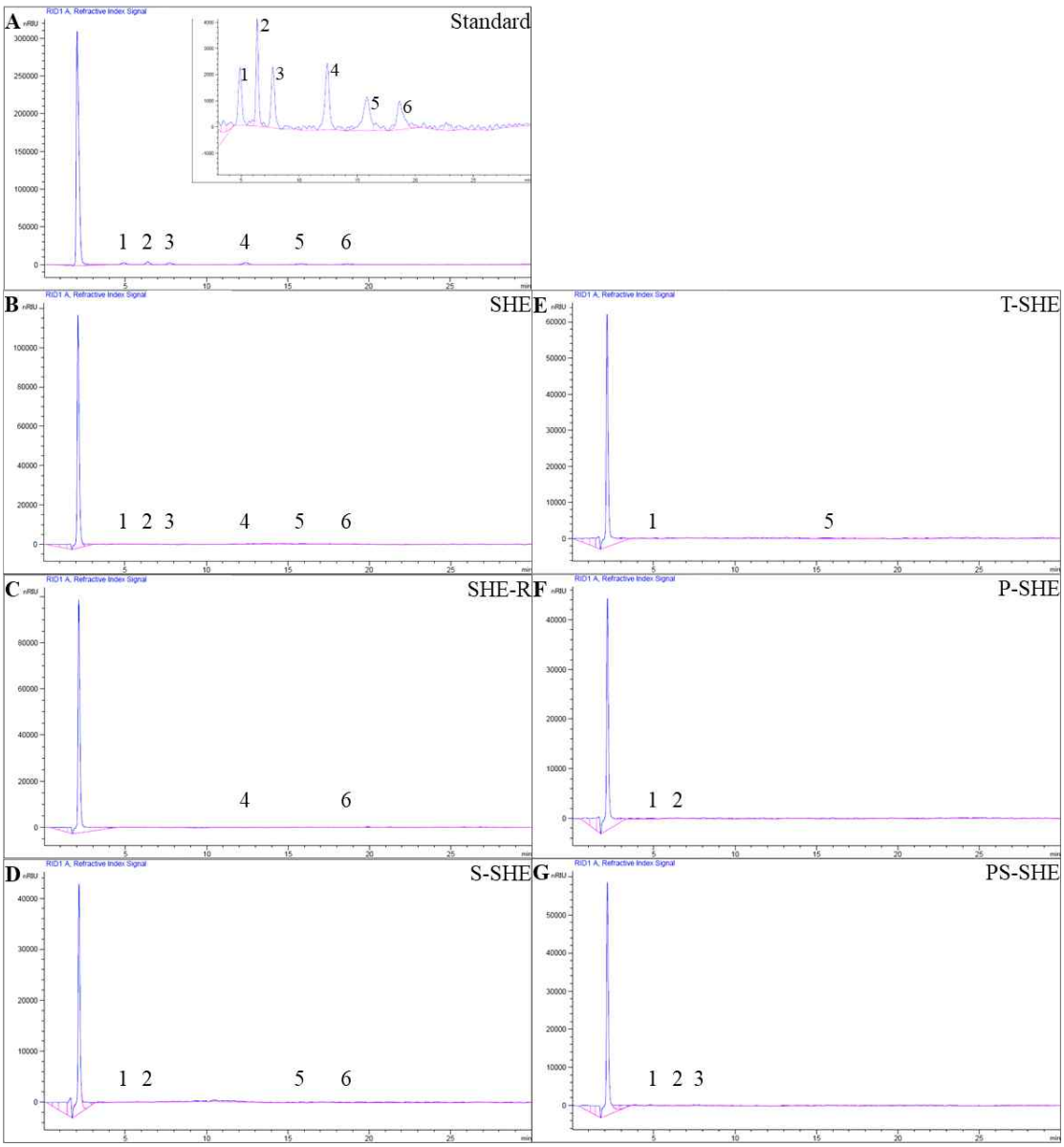

Fig. 3. HPLC-RID chromatograms of (A) standard solution of sugars, (B) SHE, (C) SHE-R, (D) S-SHE, (E) T-SHE, (F) P-SHE, and (G) PS-SHE. Peaks: fucose (1), fructose (2), glucose (3), sucrose (4), maltose (5), and lactose (6).

Table 5. Sugar contents in *S. horneri* extract and its solvent fractions

Sugar	*S. horneri* extract and its fractions (mg/100 g)					
	SHE[1)]	SHE-R	S-SHE	T-SHE	P-SHE	PS-SHE
Fucose	1.17±0.38[a2)]	-[3)]	0.65±0.02[b]	1.34±0.30[a]	0.92±0.13[ab]	1.20±0.33[a]
Fructose	1.67±0.04[a]	-	1.53±0.03[a]	-	1.50±0.01[a]	1.57±0.03[a]
Glucose	1.41±0.48[a]	-	-	-	-	1.04±0.12[a]
Sucrose	1.95±0.23[a]	1.77±0.38[a]	-	-	-	-
Maltose	0.59±0.36[a]	-	0.76±0.29[a]	0.54±0.14[a]	-	-
Lactose	3.78±0.34[a]	3.70±0.54[a]	3.73±0.24[a]	-	-	-

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) Each value is mean±standard deviation. Means with different small letters in the same row are significantly different ($p < 0.05$).

3) - means not detected.

3.7. Phenolic acid compounds

Totally 11 phenolic acid compounds, gallic acid, protocatechuic acid, catechin, vanillic acid, chlorogenic acid, caffeic acid, gentisic acid, p-coumaric acid, trans-ferulic acid, myricetin, and quercetin were identified from *S. horneri* extracts and SHE fractions. Fig. 4. shows the HPLC - DAD chromatogram of standard phenolic compounds and samples. Phenolic acid compositions of the *S. horneri* extracts and fractions of SHE are given in the Table 6.

Gallic acid was the predominant phenolic compound among eleven phenolic acids as the SHE (175.55 mg/100 g) was the highest level of gallic acid, followed by PS-SHE (155.22 mg/100 g), T-SHE (147.58 mg/100 g), and P-SHE (145.02 mg/100 g). Catechin was only detected in T-SHE at 64.42 mg/100 g and vanillic acid, a hydroxybenzoic acids was found in P-SHE as 63.16 mg/100 g. Gallic acid is a secondary metabolite that occurs naturaly in most plants. Previous studies have shown that gallic acid indicated various bioactivities including antioxidant, anti-inflammatory, anti-cancer, and anti-microbial (Fernandes and Salgado, 2016; Doğan and Akbaş, 2013). The major phenolic acid compounds found in *Sargassum* species are gallic acid and p-hydroxybenzoic acid (Hakim and Patel, 2020). Rajauria (2018) also reported predominant phenolic compounds in brown algae, *H. elongata* was phloroglucinol (394.1 μg/g) and gallic acid (96.3 μg/g). *S. scoparium* contained high amounts of gallic acid in four different solvent extracts (ethanol, methanol, aqueous methanol, and water), about 2.80, 34.42, 71.45, and 90.62 mg/100 g, respectively, indicating similar results of the current study (López et al., 2011).

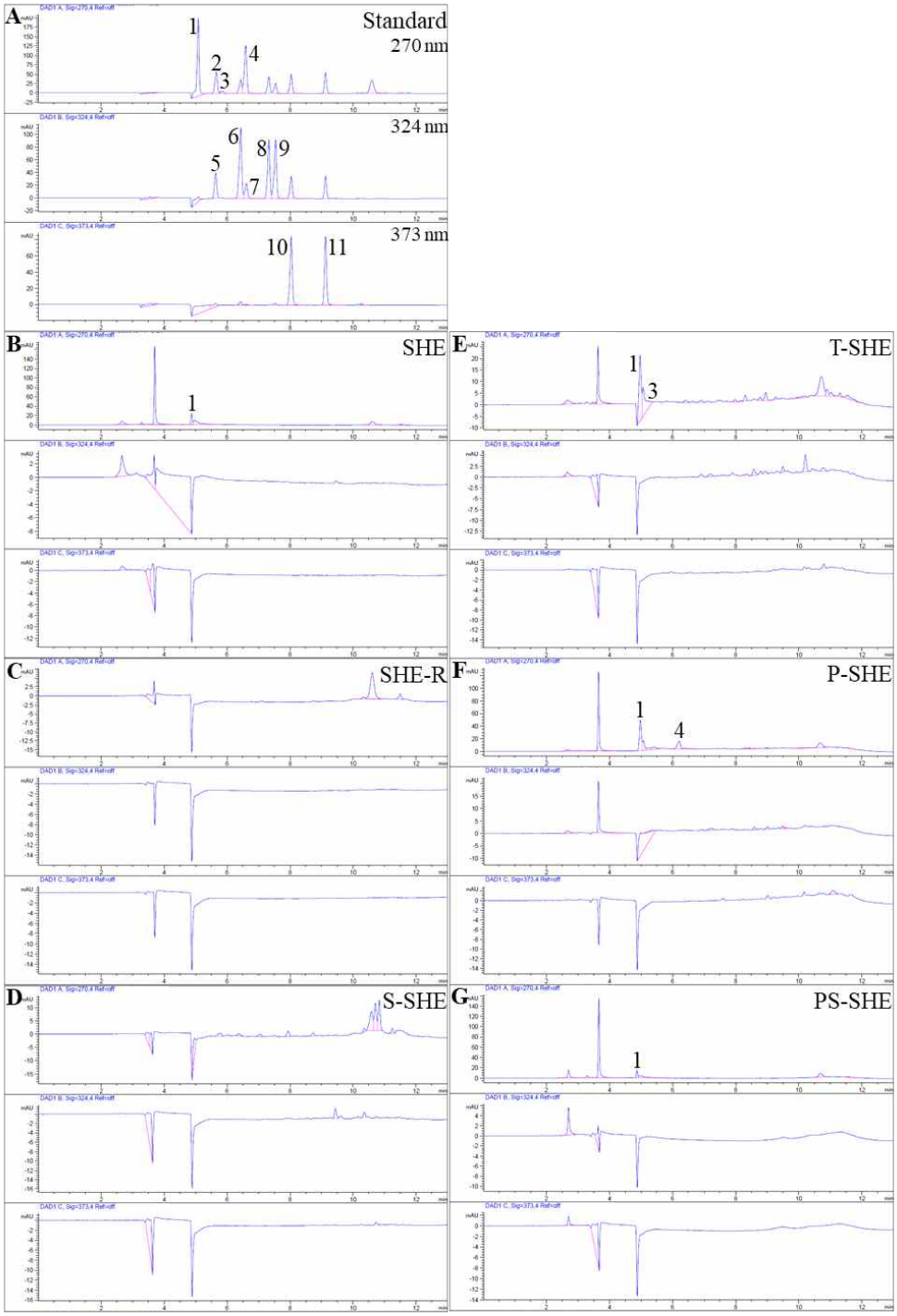

Fig. 4. HPLC-DAD chromatograms of (A) standard solution of phenolic acids, (B) SHE, (C) SHE-R, (D) S-SHE, (E) T-SHE, (F) P-SHE, and (G) PS-SHE. Peaks: gallic acid (1), protocatechuic acid (2), catechin (3), vanillic acid (4), chlorogenic acid (5), caffeic acid (6), gentisic acid (7), p-coumaric acid (8), trans-ferulic acid (9), myricetin (10), and quercetin (11).

Table 6. Phenolic acid contents in *S. horneri* extract and its solvent fractions

Phenolic acid	*S. horneri* extract and its fractions (mg/100 g)					
	SHE[1)]	SHE-R	S-SHE	T-SHE	P-SHE	PS-SHE
Gallic acid	175.55±31.22[a2)]	-	-	147.58±33.80[a]	145.02±2.46[a]	155.22±6.81[a]
Protocatechuic acid	-[3)]	-	-	-	-	-
Catechin	-	-	-	64.42±0.36[a]	-	-
Vanillic acid	-	-	-	-	63.16±3.69[a]	-
Chlorogenic acid	-	-	-	-	-	-
Caffeic acid	-	-	-	-	-	-
Gentisic acid	-	-	-	-	-	-
p-Coumaric acid	-	-	-	-	-	-
trans-Ferulic acid	-	-	-	-	-	-
Myricetin	-	-	-	-	-	-
Quercetin	-	-	-	-	-	-

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) Each value is mean±standard deviation. Means with different small letters in the same row are significantly different ($p < 0.05$).

3) - means not detected.

3.8. Identification of phenolic compounds in P-SHE

We further analyzed the phenolic acid compounds of P-SHE using UPLC-Q-TOF-MS in negative ionization modes (Table 7 and Fig. 5.). A spiked peak, peak 1, with a negative molecular ion ($[M-H]^-$) at m/z 317.0652 was detected in P-SHE (Fig. 5A.). This compound showed similar fragmentation pattern of gallic acid esterified with glucose (Table 7 and Fig. 5B.). In addition, a cleavage peak at m/z 225.01 was also observed in the MS spectra, which was consistent with the presence of gallic acid esterified with glucose ions without $C_3H_5O_3$ [M-H-$C_9H_9O_7$] (Fig. 5B.). Fragmentations of sulfate conjugates from peak 1 were observed (data not shown). These results confirm gallic acid as the major phenolic compound present in P-SHE.

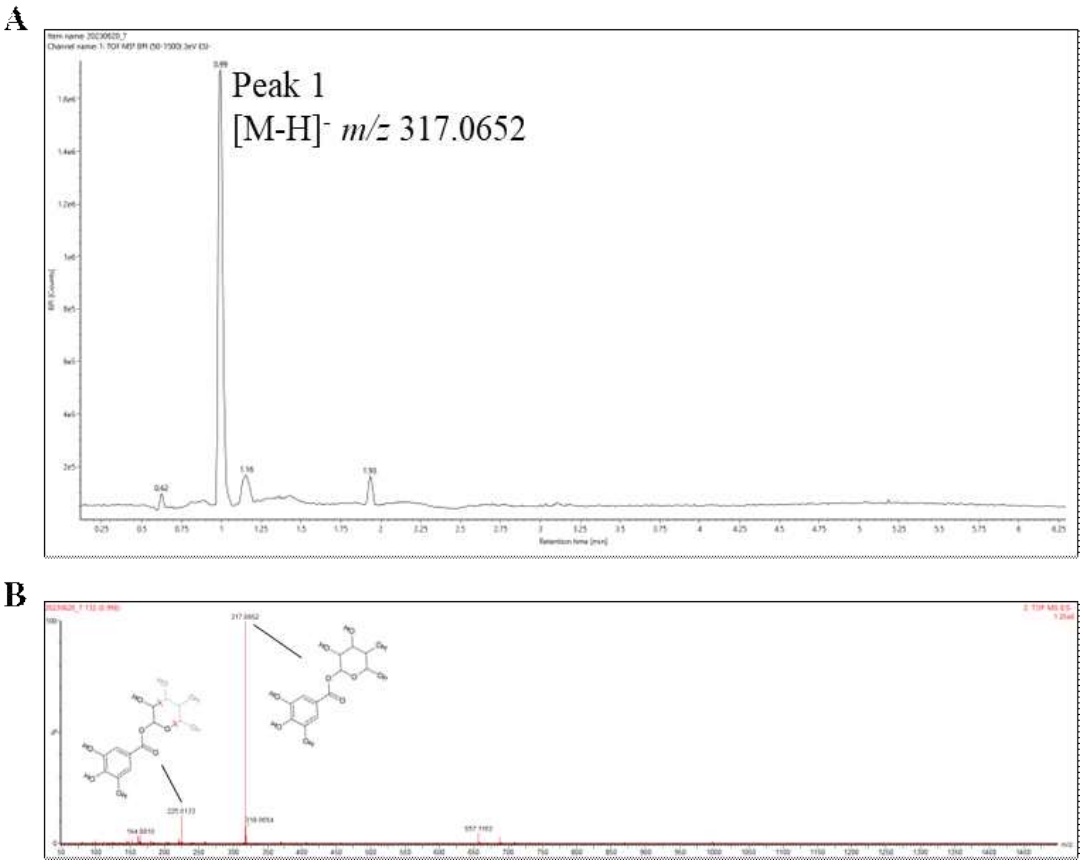

Fig. 5. (A) MS chromatogram of peak 1 identified as gallic acid esterified with glucose from P-SHE. (B) Mass spectra of (2R,3S,4R,5R,6R)-3,4,5,6-Tetrahydroxytetrahydro-2H-pyran-2-yl 3,4,5-trihydroxybenzoate with observed of m/z 317.0652 in P-SHE.

Table 7. $[M-H]^-$ data of gallic acid esterified glucose from P-SHE

Peak	Retention time (min)	Molecular weight	$[M-H]^-$, *m/z*	Molecular formula	Compounds name
1	0.99	317.0546	255.0073, 80.9659, 89.9580, 87.9597	$C_{12}H_{14}O_{10}$	(2R,3S,4R,5R,6R)-3,4,5,6-Tetrahydroxytetrahydro-2H-pyran-2-yl 3,4,5-trihydroxybenzoate

Peak 1 was the main observed fragment. Although other ions were found, they have not been included.

3.9. *In vitro* antioxidants activities

In vitro antioxidant activities including DPPH radical, $ABTS^+$ radical, hydrogen peroxide, and hydroxyl radical scavenging, ferrous ion chelating, and reducing power of *S. horneri* extracts and fractions at 1 mg/mL are shown in Table 8.

There are two antioxidant capacity methods depending on the mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET). HAT-based methods measure the hydrogen atom donating ability, while SET-based methods measure the ability of potential antioxidants to transfer electrons to reduction (Lee JH, 2010). The SET-based methods include assays such as DPPH radical and $ABTS^+$ radical scavenging assay. Other assays, such as hydrogen peroxide and hydroxyl radical scavenging also measure the ability to scavenge oxidants that are damaging to biological systems (Gulcin İ, 2020).

DPPH is a free radical, converting to the stable non-radical form DPPH-H upon accepting an electron or hydrogen radical and used widely to evaluate antioxidant activity (Marinova and Batchvarov, 2011; Yeo and Shahidi, 2019). The DPPH radical scavenging activity of the SHE and SHE-R were 41.07 and 30.80%, respectively. Among fractions, the highest DPPH radical scavenging activity was observed in the P-SHE (70.63%, $p<0.05$), followed by T-SHE (62.86%), PS-SHE (42.90%), and S-SHE (37.68%). There was a significant difference in DPPH inhibition depending on extraction solvent polarity. This result suggested that compounds with the strong radical scavenging ability in *S. horneri* may be more soluble in a slightly less polar solvent, such as chloroform and ethyl acetate.

The P-SHE and T-SHE showed high $ABTS^+$ radical scavenging activity at 43.62 and 39.89%, respectively. A comparable chemical reaction mechanism, which involves reducing the compound by a single electron transfer, is used

in both DPPH and ABTS radical scavenging assays (Ferri et al., 2013). Similar to the results of the DPPH radical scavenging activity, P-SHE showed a greater ability to scavenge free radicals than other extracts and fractions. The electron donation capacity of the compounds to radicals depends on the structural properties and concentration of antioxidants, such as flavonoids and other phenolic compounds, with the electron donation capacity of these compounds indicating an association with the antioxidant activity (Huyut et al., 2017; Hwang et al., 2021; Karahan et al., 2015). The P-SHE and T-SHE also had high TPC and TFC (Table 2), suggesting that high presence in polyphenolic and flavonoids could be correlated with their radical scavenging activity.

Hydrogen peroxide is a kind of ROS produced by oxygen molecules in cellular processes and is a relatively unreactive non-radical. However, it causes oxidative stress, which is associated with pathological conditions, such as cancer, diabetes, and cardiovascular diseases. Therefore, scavenging hydrogen peroxide using antioxidants could prevent the harmful reaction initiated by the hydroxyl radical (Aryal et al., 2019; Chen et al., 2017). The ability of antioxidants to scavenge hydrogen peroxide is inhibited by directly reacting with hydrogen peroxide or through reacting with intermediates produced by the enzyme and hydrogen peroxide (Martínez-Tomé et al., 2001). The hydrogen peroxide scavenging activity of P-SHE was the greatest as 74.88%, which was very strong compared to those of S-SHE (7.44%) and PS-SHE (11.28%). Previous study showed the greater hydrogen peroxide scavenging activity in the ethyl acetate fraction of the brown algae, *T. conoides* (IC_{50} 1.49 mg/mL), supporting that the polar fractions of brown seaweeds contain a rich source of natural antioxidants (Chakraborty K et al., 2013).

Hydroxyl radicals are highly reactive and regarded as harmful oxidants for living organisms. Hydroxyl radicals cause oxidative damage to DNA, lipids,

and proteins, resulting in damage to biomolecules. Therefore, to protect living organisms, the reduction of hydroxyl radicals is essential (Su and Li, 2020). The Fenton reaction produces the hydroxyl radical when hydrogen peroxide reacts with ferrous. This radical then reacts with deoxyribose, generating a chromogen as it combines with thiobarbituric acid. When heated, the radical scavenger competes with the hydroxyl radical, limiting the formation of the chromogen (MacDonald-Wicks et al., 2006). All *S. horneri* extract and their solvent fractions have the activity of scavenging hydroxyl radicals. The greatest hydroxyl radical scavenging activities were S-SHE (31.85%) and T-SHE (31.61%), followed by P-SHE (27.65%), SHE-R (25.78%), SHE (15.53%), and PS-SHE (14.94%).

Ferrous ions are the most powerful pro-oxidants of the various species of transition metals found in food systems (Rajauria, 2019). Antioxidants bind with a certain ferrous ion, whereas the remaining ferrous ions could react with ferrozine, forming stable and dark purple iron ion-ferrozine complexes (Santos et al., 2017). Ferrous ion chelating effect of *S. horneri* and their solvent fractions, T-SHE showed the highest Fe^{2+} chelating activity followed by P-SHE, S-SHE, SHE, PS-SHE, and SHE-R. In the case of solvent fractions, except PS-SHE, showed more than 90% Fe^{2+} chelating activity at 1 mg/mL. A similar result was reported that metal ion chelating effects of solvent fractions from *G. acerosa*, ethyl acetate fractions showed significantly higher metal chelating activity of 99.02% at 0.05 mg/mL (Suganthy et al., 2013). Brown seaweeds including *Sargassum* spp have potential metal chelators due to the presence of phenolics (Senevirathne et al., 2006). The metal ion chelating activity of phenolic compounds depends on their particular phenolic structure and the number and location of the hydroxyl groups (Santoso et al., 2004). The different extracts and fractions of *S. horneri* showed exhibition for metal ion binding capacity, suggesting the correlation between Fe^{2+} binding capacity and phenolic compounds.

The reducing power suggests that the antioxidant compounds are electron donors that could reduce the oxidized intermediates (Surendraraj et al., 2013). Antioxidants reduce the Fe^{3+}-ferricyanide complex to the ferrous. When $FeCl_3$ is added to the ferrous, it forms a Prussian blue complex that can be measured at 700 nm to determine the amount of reduction (Gulcin et al., 2002). In the present study, the highest reducing power was observed in P-SHE (0.37, as indicated by the absorbance at 700 nm). Farvin and Jacobsen (2013) have reported that reducing power of the ethanol extracts from brown algae, *S. muticum* showed 0.4 at 1,000 μg/mL. Extracts containing high levels of total phenolic content were observed to be potent in reducing ions, which indicates that polyphenols could be the main components responsible for the reducing properties of the extracts.

The results of the antioxidant activities indicate that SHE fractions showed higher antioxidant activities than those of extracts and especially, ethyl acetate fraction (P-SHE) and chloroform (T-SHE) fraction acted as the best antioxidant among four different solvent fractions. P-SHE and T-SHE were polyphenols and terpenoids rich fractions which mostly tended to high in antioxidant activities (Faraone et al., 2018).

Furthermore, the correlation between TPC, TFC, and *in vitro* antioxidant activities of SHE, SHE-R, and SHE fractions are shown in Table 9. TPC showed a significantly high positive correlation with RP (r^2=0.992), DPPH (r^2=0.985), and ABTS (r^2=0.948) (p<0.01). On the other hand, the correlation coefficients of TPC and H_2O_2, ·OH, and FC were relatively low as 0.331, 0.331, and 0.584, respectively. TFC also significantly high positive correlation with ABTS (r^2=0.973, p<0.01). DPPH, FC, and RP of r^2 values were 0.845, 0.915, and 0.817, respectively (p<0.05), whereas H_2O_2 and ·OH showed no correlation. Several studies have shown that the antioxdiant activity of brown seaweed was correlated with phenols. Tana et al. (2021) reported that the correlation between TPC and antioxidant activity of correlation coefficient was

more than 0.9 for *S. asperum* extracts, indicating a strong relationship. Pinteus et al. (2017) found a positive correlation between TPC of seaweed extracts and their scavenging capacity on DPPH and peroxyl radicals.

Table 8. *In vitro* antioxidant activities of *S. horneri* extract and its solvent fractions

S. horneri extract and its fractions (1 mg/mL)	DPPH (%)[3]	ABTS (%)	H_2O_2 (%)	·OH (%)	FC (%)	RP
SHE[1]	41.07±0.75^{d}[4]	34.22±1.57^{c}	9.36±4.06^{d}	15.53±2.62^{c}	53.54±6.59^{c}	0.18±0.01^{c}
SHE-R	30.80±1.06^{f}	32.85±1.14^{c}	15.83±2.45^{c}	25.78±3.26^{b}	17.42±2.47^{d}	0.18±0.01^{c}
S-SHE	37.68±1.33^{e}	36.42±2.69^{bc}	7.44±2.41^{d}	31.85±2.94^{a}	93.29±3.38^{b}	0.17±0.00^{c}
T-SHE	62.86±0.87^{b}	39.89±3.52^{ab}	45.55±6.01^{b}	31.61±2.31^{a}	106.50±3.05^{a}	0.25±0.03^{b}
P-SHE	70.63±0.35^{a}	43.62±3.16^{a}	74.88±2.69^{a}	27.65±3.38^{ab}	94.18±5.62^{b}	0.37±0.01^{a}
PS-SHE	42.90±0.58^{c}	34.20±1.59^{c}	11.28±4.61^{d}	14.94±4.74^{c}	22.14±2.14^{d}	0.20±0.01^{c}
Positive control (0.1 mg/mL)[2]	98.64±0.19	101.26±0.06	99.75±0.77	77.24±0.41	107.35±0.20	1.35±0.03

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) Ascorbic acid was used a positive control for DPPH, $ABTS^{+}$, H_2O_2 scavenging activity and reducing power. EDTA was used as a positive control for ferrous ion chelating effect and BHT was used as a positive control for ·OH scavenging activity.

3) DPPH: DPPH free radical scavenging activity, ABTS: $ABTS^{+}$ radical scavenging activity, H_2O_2: Hydrogen peroxide scavenging activity, ·OH: Hydroxyl radical scavenging activity, FC: Ferrous ion chelating effect, and RP: Reducing power effect (absorbance at 700 nm).

4) Each value is mean±standard deviation. Means with different small letters in the same column in all sample are significantly different ($p< 0.05$).

Table 9. Pearson correlation coefficient of total polyphenol contnets, total flavonoid contents and *in vitro* antioxidant activities

Factor	TPC[1)]	TFC	DPPH	ABTS	H_2O_2	·OH	FC	RP
TPC	1	.861[*2)]	.985[**]	.948[**]	.331	.331	.584	.992[**]
TFC		1	.845[*]	.973[**]	.611	.611	.915[*]	.817[*]
DPPH			1	.929[**]	.419	.419	.570	.983[**]
ABTS				1	.534	.534	.802	.924[**]
H_2O_2					1	1.000[**]	.707	.336
OH·						1	.707	.336
FC							1	.521
RP								1

1) TPC: total polyphenol contents, TFC: total flavonoid contents, DPPH: DPPH free radical scavenging activity, ABTS: $ABTS^+$ radical scavenging activity, H_2O_2: Hydrogen peroxide scavenging activity, ·OH: Hydroxyl radical scavenging activity, FC: Ferrous ion chelating effect, and RP: Reducing power effect.

2) Significance was determined using SPSS by Pearson's correlation coefficient, *$p<0.05$, **$p<0.01$.

3.10. *In vitro* antioxidants activities in PM-induced oxidative damage

Particulate matter (PM) causes oxidative stress upon exposure and antioxidant activities of SHE, SHE-R, and SHE fractions could be changed against PM-induced oxidative stress.

In vitro antioxidant activities of SHE, SHE-R, and SHE fractions at 1 mg/mL when treated with PM at 0.125 mg/mL are shown in Table 10.

The DPPH free radical scavenging activities of SHE, T-SHE, P-SHE, and PS-SHE in PM-induced oxidation were decreased when compared to those without PM treatment. Particularly, P-SHE and T-SHE showed significant decrease of DPPH free radical scavenging activities from 70.63 to 55.10% and from 62.86 to 51.17% after PM-induced oxidation (Table 8 and Table 10). However, their radical scavenging activities against PM-induced oxidative stress still showed high levels compared to other extracts or fractions.

The $ABTS^+$ radical scavenging activities of SHE fractions were slightly decreased against PM-induced oxidation when compared to those without PM (Table 8). There were no significant differences in the $ABTS^+$ radical scavenging activities of SHE and SHE-R. DPPH free radical and $ABTS^+$ radical scavenging activity methods can effectively measure antioxidant activity for bioactive components (Gülçin et al., 2010). These results indicated that SHE fractions has a potential antioxidative activity to reduce radicals when PM-induced oxidation.

The hydrogen peroxide activity of PM-induced SHE and SHE-R were 8.83, and 5.33%, respectively, which were reduced compared to those without PM treatment. Among fractions, P-SHE and T-SHE greatly scavenged H_2O_2 and their activities reduced from 74.88 to 44.17% and from 45.55 to 18.96%, respectively, against PM-induced oxidation. On the other hand, the H_2O_2 scavenging activities of SHE, SHE-R, S-SHE, and PS-SHE showed lower values, with P-SHE and T-SHE still showing higher scavenging ability.

Therefore, polyphenol-rich and terpenoid-rich fractions more impacted on the H_2O_2 scavenging activities.

Hydroxyl radical scavenging activities of SHE, SHE-R, and SHE fractions were decreased after PM treatment. Particularly, SHE-R, S-SHE, and T-SHE greatly reduced hydroxyl radical scavenging activities. Depending on major bioactive compounds in fractions, the antioxidant activities were differently changes when treated with PM.

Many radical reactions could be generated from ferrous iron (Fe^{2+}) due to its ability to transfer single electrons (Liu et al., 2021). Therefore, measuring iron chelation may be an effective method of assessing antioxidant capacity. When treated with PM, the ferrous ion chelating effect of SHE, S-SHE, T-SHE, P-SHE, and PS-SHE with PM (0.125 mg/mL) was decreased, from 53.54 to 18.35, 93.29 to 18.35, 106.50 to 19.51, 94.18 to 18.24 and 22.14 to 16.23%, respectively. The presence of low concentration PM extremely reduced the ferrous ion chelating effect on S-SHE, T-SHE, and P-SHE.

The reducing power of *S. horneri* extract and its fractions with PM (0.125 mg/mL) were decreased from 0.17-0.37 to 016-0.18 (absorbance at 700 nm) after PM treatment. The decreased antioxidant activities may be due to lipid peroxidation, induced by PM inhibiting free radical scavenging mechanisms or metal chelating mechanisms.

PM-induced oxidation and *in vitro* antioxidant activities of SHE, SHE-R, and SHE fractions were overall decreased. Nevertheless, in terms of radical scavenging activity and chelating effect, *S. horneri* extract and its fractions showed antioxidant capacity against PM-induced oxidative stress. These results indicate that SHE, SHE-R, and SHE fractions have a great potential for antioxidant activities and delay the oxidative stress caused by PM.

Table 10. *In vitro* antioxidant activities of *S. horneri* extract and its solvent fractions induced by particulate matter at 0.125 mg/mL

S. horneri extract and its fractions (1 mg/mL)	DPPH (%)[2)]	ABTS (%)	H_2O_2 (%)	·OH (%)	FC (%)	RP
SHE[1)]	39.57±1.19^{c}[3)]	34.34±0.16^{c}	8.83±0.92^{c}	9.70±1.68^{c}	18.35±1.54^{a}	0.17±0.01^{a}
SHE-R	36.52±0.65^{d}	32.51±0.08^{d}	5.33±2.37^{c}	9.63±1.62^{c}	17.78±0.89^{b}	0.16±0.01^{b}
S-SHE	39.71±1.35^{c}	32.74±0.23^{d}	4.98±0.90^{c}	9.82±1.66^{c}	18.35±1.28^{a}	0.16±0.01^{b}
T-SHE	51.17±0.32^{b}	35.72±0.09^{b}	18.96±2.03^{b}	13.83±2.00^{b}	19.51±0.36^{a}	0.18±0.00^{a}
P-SHE	55.10±0.17^{a}	36.43±0.01^{a}	44.17±5.67^{a}	17.58±1.58^{a}	18.24±0.84^{a}	0.18±0.00^{a}
PS-SHE	39.91±1.64^{c}	34.09±0.13^{c}	16.37±4.80^{b}	12.11±1.47^{bc}	16.23±0.65^{ab}	0.18±0.00^{a}

1) SHE: *S. horneri* ethanol extract, SHE-R: SHE residue, S-SHE: n-hexane fraction, T-SHE: chloroform fraction, P-SHE: ethyl acetate fraction, and PS-SHE: water fraction.

2) DPPH: DPPH free radical scavenging activity, ABTS: $ABTS^{+}$ radical scavenging activity, H_2O_2: Hydrogen peroxide scavenging activity, ·OH: Hydroxyl radical scavenging activity, FC: Ferrous ion chelating effect, and RP: Reducing power effect (absorbance at 700 nm).

3) Each value is mean±standard deviation. Means with different small letters in the same column in all sample are significantly different ($p< 0.05$).

4. Conclusion

This study was conducted to determine the structural characterization of bioactive components in *Sargassum horneri* ethanol extracts and their solvent fractions. Extraction yields were high in the order of S-SHE (75.35%) T-SHE (13.41%), P-SHE (2.46%), and PS-SHE (1.07%). To screen major bioactive compounds, TPC, TFC, sulfate group contents, and reducing sugar were measured. TPC and TFC tended to be higher in SHE fractions than those in SHE and SHE-R, while sulfate group contents were more found in SHE-R and PS-SHE. Method validation of HPLC analysis for sterols, simple sugars, and phenolic acids showed excellent correlation coefficients, $R^2 \geq 0.99$. The predominant sterol in SHE, SHE-R, and SHE fractions was fucosterol, while six simple sugars (fucose, fructose, glucose, sucrose, maltose, and lactose) were variously found in SHE fractions. Among eleven phenolic compounds, gallic acid was the most abundant. Gallic acid esterified with glucose ions was isolated and identified in P-SHE. For *in vitro* antioxidant activities, SHE fractions, especially T-SHE and P-SHE showed high in ferrous ion chelating effect, DPPH free radical, hydrogen peroxide scavenging activity, and reducing power. The antioxidant activities were also determined in PM at 0.125 mg/mL, which induced oxidative damage. The antioxidant activities of fractions tended to decrease with PM treatment. Nevertheless, SHE, SHE-R, and SHE fractions which have different types of bioactive compounds showed high antioxidant activities, indicating its potential as a natural antioxidant in the food and pharmaceutical industries.

REFERENCES

Abdul QA, Choi RJ, Jung HA, Choi JS. Health benefit of fucosterol from marine algae: A review. Journal of the Science of Food and Agriculture. 96: 1856 - 1866 (2016)

Aminina NM, Karaulova EP, Vishnevskaya TI, Yakush E V, Kim YK, Nam KH, Son KT. Characteristics of polyphenolic content in brown algae of the Pacific coast of Russia. Molecules. 25: 3909 (2020)

Association of Official Analytical Chemists. AOAC guidelines for single laboratory validation of chemical analytical method (2002)

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants. 8: 96 (2019)

Bak JH, Miyazaki Y, Nakano H, Matsui T. Profiling sulfate content of polysaccharides in seaweed species using a ligand-assisted 1H-NMR asay. Food Science and Technology Research. 27: 505-510 (2021)

Bogolitsyn K, Dobrodeeva L, Druzhinina A, Ovchinnikov D, Parshina A, Shulgina E. Biological activity of a polyphenolic complex of Arctic brown algae. Journal of Applied Phycology. 31: 3341 - 3348 (2019)

Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. Journal of Food Science and Technology. 52: 1924 - 1935 (2015)

Chakraborty K, Praveen NK, Vijayan KK, Rao GS. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaepphyta, Sargassaceae) collected from Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine. 3: 8-16 (2013)

Chen S, Sathuvan M, Zhang X, Zhang W, Tang S, Liu Y, Cheong KL.

Characterization of polysaccharides from different speciecs of brown seaweed using saccharide mapping and chromatographic analysis. BMC Chemistry. 15: 1 (2021)

Chen Y, Shi X, Lu Z, Wang X, Wang Z. A fluorescent probe for hydrogen peroxide in vivo based on the modulation of intramolecular charge transfer. Analytical Chemistry. 89: 5278-5284 (2017)

Cho ML, Lee HS, Kang IJ, Won MH, You SG. Antioxidant properties of extract and fractions from *Enteromorpha prolifera*, a type of green seaweed. Food Chemistry. 127: 999 - 1006 (2011)

Cho SM, Yang HJ, Jeon YJ, Lee JC, Jin YH, Baek NI, Kim DS, Kang SM, Yoon MS, Yong HI, Shimizu M, Han DS. Phlorotannins of the edible brown seaweed *Ecklonia cava* Kjellman induce sleep via positivie allosteric modulation of gamma-aminobutyric acid type A-benzodizepine recptor: A novel neurological activitiy of seawwed polyphenols. Food Chemistry. 132: 1133-1142 (2012)

Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RBG, Nobre LTDB, Costa MSSP, Almeida-Lima J, Farias EHC, Leite EL, Rocha HAO. Biological activities of sulfated polysaccharides from tropical seaweeds. Biomedicine and Pharmacotherapy. 64: 21 - 28 (2010)

Cvitković D, Dragović-Uzelac V. Dobrinčic A, Čož-Rakovac R, Balbino S. The effect of solvent and extraction method on the recovery of lipid fractionfrom Adriatic Sea macroalgae. Algal Research. 56: 102291 (2021)

Dang TT, Bowyer MC, Van Altena IA, Scarlett CJ. Comparison of chemical profile and antioxidant properties of the brown algae. International Journal of Food Science and Technology. 53: 174 - 181 (2018)

Dave N, Varadvaenkatesan T, Singh RS, Giri BS, Selvaraj R, Vinayagam R. Evaluation of seasonal variation and the optimization of reducing sugar extraction form *Ulva prolifera* biomass using thermochemical method. Environmental Science and Pollution Research. 28: 58857-58871 (2021)

Dehkharghanian M, Adenier H, Vijayalakshmi MA. Analytical methods study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. Food Chemistry. 121: 863-870 (2010)

Dobrinčić A, Balbino S, Zorić Z, Pedisić S, Kovačević DB, Garofulić IE, Dragović-Uzelac V. Advanced technologies for the extraction of marine brown algal polysaccharides. Marine Drugs. 18: 3 (2020)

Doğan HH and Akbaş G. Biological activity and fatty acid composition of Caesar's mushroom. Pharmaceutical Biology. 51: 863 - 871 (2013)

Eom SH, Kim YM, Kim SK. Antimicrobial effect of phlorotannins from marine brown algae. Food and Chemical Toxicology. 50: 3251 - 3255 (2012)

Faraone I, Rai DK, Chiummiento L, Fernadez E, Choudhary A, Prinzo F, Milella L. Antioxidant activity and phytochemical characterization of *Senecio clivicolus* Wedd. Molecules. 23: 2497 (2018)

Farvin KHS and Jacobsen C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chemistry. 138: 1670 - 1681 (2013)

Fernandes FHA and Salgado HRN. Gallic acid: Review of the methods of determination and quantification. Critical Reviews in Analytical Chemistry. 46: 257 - 265 (2016)

Fernando IPS, Ryu BM, Ahn G, Yeo IK, Jeon YJ. Therapeutic potential of algal natural products against metabolic syndrome: A review of recent developments. Trends in Food Science and Technology. 97: 286 - 299 (2020)

Ferri M, Gianotti A, Tassoni A. Oprimisation of assay conditions for the determination of antioxidant capacitiy and polyphenols in cereal food components. Journal of Food Composition and Analysis. 30: 94-101 (2013)

Filonchyk M, Yan H, Yang S, Hurynovich V. A study of $PM_{2.5}$ and PM_{10} concentrations in the atmosphere of large cities in Gansu Province, China, in summer period. Journal of Earth System Science. 125: 1175-1187 (2016)

Franco D, Sineiro J, Rubilar M, Sánchez M, Jerez M, Pinelo M, Costoya N, Núñez MJ. Polyphenols from plant materials: extraction and antioxidant power. Electronic Journal of Environmental, Agricultural and Food Chemistry. 7: 3210-3216 (2008)

García-Ríos V, Ríos-Leal E, Robledo D, Freile-Pelegrin Y. Polysaccharides composition from tropical brown seaweeds. Phycological Research. 60: 305-315 (2012)

Gómez-Guzmán M, Rodríguez-Nogales A, Algieri F, Gálvez J. Potential role of seaweed polyphenols in cardiovascular-associated disorders. Marine Drugs. 16: 250 (2018)

Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Archves of Toxicology. 94: 651-715 (2020)

Gülçin İ. Antioxidant activity of food constituents: An overview. Archives of Toxicology. 86: 345 - 391 (2012)

Gulcin İ, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. On the in vitro antioxidative properties of melatonin. Juournal of Pineal Research. 33: 167-171 (2002)

Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein H. Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry. 3: 43-53 (2010)

Hakim MM and Patel IC. A review on phytoconstituents of marine brown algae. Future Journal of Pharmaceutical Sciences. 6: 129 (2020)

Hannan MA, Sohag AAM, Dash R, Haque MN, Mohibbullah M, Oktaviani DF, Hossain MT, Choi HJ, Moon IS. Phytosterols of marine algae: Insights into the potential health benefits and molecular pharmacology. Phytomedicine. 69: 153201 (2020)

Heo SJ, Cha SH, Lee KW, Cho SK, Jeon YJ. Antioxidant activities of Chlorophyta and Phaeophyta from Jeju Island. Algae. 20: 251 - 260 (2005)

Herath KHINM, Kim HJ, Jang JH, Kim HS, Kim HJ, Jeon YJ, Jee YH.

Mojabanchromanol isolated from *Sargassum horneri* attenuates particulate matter induced inflammatory responses via suppressing TLR2/4/7-MAPK signaling in MLE-12 cells. Marine Drugs. 18: 355 (2020)

Hikihara R, Yamasaki Y, Shikata T, Nakayama N, Sakamoto S, Kato S, Hatate H, Tanaka R. Analysis of phytosterol, fatty acid, and carotenoid composition of 19 microalgae and 6 bivalve species. Journal of Aquatic Food Product Technology. 29: 461 - 479 (2020)

Huyut Z, Beydemir Ş, Gülçin I. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochemistry Research International. 2017 (2017)

Hwang EJ, Lee YG, Lee HJ, Cho JY, Moon JH. Major constituents and antioxidant activities of domestic onion (*Allium cepa L.*) cultivars. Korean Journal of Food Science and Technology. 53: 434 - 445 (2021)

Kaneria M, Bapodara MB, Chanda SV. Effect of extraction techniques and solvents on antioxidant activity of Pomegranate (*Punica granatum L.*) leaf and stem. Food Analytical Methods. 5: 396-404 (2012)

Karahan F, Kulak M, Urlu E, Gözüacik HG, Böyümez T, Şekeroğlu NI, Doganturk IH. Total phenolic content, ferric reducing and DPPH scavenging activity of *Arum dioscoridis*. Natural Product Research. 29: 1678 - 1683 (2015)

Kim BM, Jun JY, Park YB, Jeon IH. Antioxidant activity of methanolic extracts from seaweeds. Journal of the Korean Society of Food Science and Nutrition. 35: 1097 - 1101 (2006)

Kim HI, Kim DS, Jung YU, Sung NY, Kim MJ, Han IJ, Nho EY, Hong JH, Lee JK, Boo MN, Kim HL, Baik SY, Jung KO, Lee SH, Kim CS, Park JB. Immune-enhancing effect of *Sargassum horneri* on cyclophosphamide-induced immunosuppression in BALB/c mice and primary cultured splenocytes. Molecules. 27: 8253 (2022)

Kim YD, Mahinda S, Koh KS, Jeon YJ, Kim SH. Reactive oxygen species

scavenging activity of Jeju native citrus peel during maturation. Journal of the Korean Society of Food Science and Nutrition. 38: 462 - 469 (2009)

Kim HS, Sanjeewa KKA, Fernando IPS, Ryu BM, Yang HW, Ahn GN, Kang MC, Heo SJ, Je JG, Jeon YJ. A comparative study of *Sargassum horneri* Korea and China strains collected along the coast of Jeju island South Korea: Its components and bioactive properties. Algae. 33: 341 - 349 (2018)

Kim JY and Yi YH. pH, acidity, color, amino acids, reducing sugars, total sugars, and alcohol in puffed millet powder containing millet Takju during fermentation. Korean Journal of Food Science and Technology. 42: 727 - 732 (2010)

Klran TR, Otlu O, Karabulut AB. Oxidative stress and antioxidants in health and disease. Journal of Laboratory Medicine. 47: 1 - 11 (2023)

Lee KH, Senevirathne M, Ahn CB, Je JY. Biological compounds extracted from codium fragile by enzymatic hydrolysis and their biological activities. Journal of the Korean Society of Food Science and Nutrition. 39: 953 - 959 (2010)

Li YX, Wijesekara I, Li Y, Kim SK. Phlorotannins as bioactive agents from brown algae. Process Biochemistry. 46: 2219 - 2224 (2011)

Libalova H, Milcova A, Cervena T, Vrbova K, Rossnerova A, Novakova Z, Topinka J, Rossner P. Kinetics of ROS generation induced by polycyclic aromatic hydrocarbons and organic extracts from ambient air particulate matter in model human lung cell lines. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 827: 50-58 (2018)

Liu M, Feng Z, Luan X, Chu W, Zhao H, Zhao G. Accelerated Fe^{2+} regeneration in an effective electro-fenton process by boosting internal electron transfer to a nitorgen-conjugated Fe(Ⅲ) complex. Environmental Science and Technology. 55: 6042-6051 (2021)

Lopes G, Sousa C, Bernardo J, Andrade PB, Valentão P, Ferreres F, Mouga T. Sterol profiles in 18 macroalgae of the Portuguese coast. Journal of

Phycology. 47: 1210 - 1218 (2011)

López A, Rico M, Rivero A, Tangil de MS. The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. Food Chemistry. 125: 1104 - 1109 (2011)

MacDonald-Wicks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant acapacity *in vitro*: a review. Journal of the Science of Food and Agriculture. 86:2046-2056 (2006)

Marinova G and Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science. 17: 11 - 24 (2011)

Martínez-Tomé M,. García-Carmona F, Murcia MA. Comparison of the antioxidant and pro-oxidant activities of broccoli amino acids with those of common food additives. Journal of the Science of Food and Agriculture. 81: 1019-1026 (2021)

Meillisa A, Woo HC, Chun BS. Production of monosaccharides and bio-active compounds derived from marine polysaccharides using subcritical water hydrolysis. Food Chemistry. 147: 70-77 (2015)

Michalak I and Chojnacka K. Algae as production systems of bioactive compounds. Engineering in Life Sciences. 15: 160 - 176 (2015)

Milović S. Stanković I, Nikolić D, Radović J, Kolundžić M, Nikolić V, Stanojković T, Petović S, Kundaković-Vasović T. Chemical analysis of selected seaweeds and seagrass from the Adriatic coast of Montenegro. Chemistry and Biodiversity. 16 (2019)

Mohd Fauziee NA, Chang LS, Wan Mustapha WA, Md Nor AR, Lim SJ. Functional polysaccharides of fucoidan, laminaran and alginate from Malaysian brown seaweeds (S*argassum polycystum*, *Turbinaria ornata* and *Padina boryana*). International Journal of Biological Macromolecules. 167: 1135 - 1145 (2021)

Mori I, Sun Z, Ukachi M, Nagano K, McLeod CW, Cox AG, Nishikawa M.

Development and certification of the new NIES CRM 28: Urban aerosols for the determination of multielements. Analytical and Bioanalytical Chemistry. 391: 1997 - 2003 (2008)

Møller P, Danielsen P, Karottki D, Jantzen K, Roursgaard M, Klingberg H, Jensen D, Christophersen D, Hemmingsen J, Cao Y, Loft S. Oxidative stress and inflammation generated DNA damage by exposure to air pollutioin particles. Mutation Research/Reviews in Mutation Research. 762: 133-166 (2014)

Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seed. Brazilian Journal of Pharmaceutical Science. 56 (2020)

Offei F, Mensah M, Thygesen A, Kemausuor F. Seaweed bioethanol production: a process selection review on hydrolysis and fermentation. Fermentation. 4: 99 (2018)

Osório C, Machado S, Peixoto J, Bessada S, Pimentel FB, Alves RC, Oliveira MBPP. Pigments content (chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. Separations. 7: 1 - 14 (2020)

Ozougwu JC. The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research in Pharmacy and Biosciences. 3: 1 - 8 (2016)

Park NY, Kim IS, Jeong YJ. Effect of extraction condition on the componential extraction of brown seaweed (*Undaria pinnatifida*). Journal of Food Science and Nutrition. 13: 321-326 (2008)

Patterson WG. The distribution of sterols in algae. Lipids. 6: 120 - 127 (1971)

Pinteus S, Silva J, Alves C, Horta A, Fino N, Rodirgues AI, Mendes S, Pedrosa R. Cyoprotective effect of seaweeds with high antioxidant activity from the Peniche coast (Portugal). Food Chemistry. 218: 591-599 (2017)

Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative

stress mitigation by antioxidants – An overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry. 209: 112891 (2021)

Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: The interplay. BioMed Research International. 2014: 761264 (2014)

Rajauria G. Optimization and validation of reverse phase HPLC method for qualitative and quantitative assessment of polyphenols in seaweed. Journal of Pharmaceutical and Biomedical Analysis. 148: 230 – 237 (2018)

Rajauria G. In-vitro antioxidant properties of lipophilic antioxidant compounds from 3 brown seaweed. Antioxidants. 8: 596 (2019)

Ruiz G, Gloaguen V, Granet R, Roy A, Mouradi-Givernaud A, Louvet F, Krausz P. Hydrophobic plastics films synthesis by mean of agaroid acylation with lauroyl chloride in the N,N-dimethylacetamide homogeneous system. Journal of Applied Phycology. 19: 1 – 13 (2007)

Sánchez-Machado DI, López-Hernández J, Paseiro-Losada P, López-Cervantes J. An HPLC method for the quantification of sterols in edible seaweeds. Biomedical Chromatography. 18: 183 – 190 (2004)

Santos JS, Brizola VRA, Granato D. High-throughtput assay comparison and standardization for metal chelating capacity screening: A proposal and application. Food Chemistry. 214: 515-522 (2017)

Santoso J, Yoshie-Stark Y, Suzuki T. Anti-oxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. Fisheries Science. 70: 183 – 188 (2004)

Saraswati, Giriwono PE, Iskandriati D, Tan CP, Andarwulan N. *Sargassum* seaweed as a source of anti-inflammatory substances and the potential insight of the tropical species: A review. Marine Drugs. 17 (2019)

Senevirathne M, Kim SH, Siriwardhana N, Ha JH, Lee KW, Jeon YJ. Antioxidant potential of *Ecklonia cava* on reactive oxygen species

scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Science and Technology International. 12: 27 - 38 (2006)

Shao P, Chen X, Sun P. Chemical characterization, antioxidant and antitumor activity of sulfated polysaccharide from *Sargassum horneri*. Carbohydrate Polymers. 105: 260 - 269 (2014)

Sirbu R, Negreanu-Pirjol T, Mirea M, Negreanu-Pirjol BS. Bioactive compounds from three green algae species along Romanian Black sea coast with therapeutically properties. European Journal of Natural Sciences and Medicine. 3: 1 (2020)

Son ES, Park JW, Kim YJ, Jeong SH, Hong JH, Kim SH, Kyung SY. Effects of antioxidants on oxidative stress and inflammatory responses of human bronchial epithelial cells exposed to particulate matter and cigarette smoke extract. Toxicology in Vitro. 67: 104883 (2020)

Su Y and Li L. Structural characterization and antioxidant activity of polysaccharide from four auriculariales. Carbohydrate Polymers. 229: 115407 (2020)

Suganthy N, Nisha SA, Pandian SK, Devi KP. Evaluation of *Gelidiella acerosa*, the red algae inhabiting South Indian coastal area for antioxidant and metal chelating potential. Biomedicine and Preventive Nutrition. 3: 399 - 406 (2013)

Sung HM, Seo YS, Yang EJ. Anti-oxidant and anti-inflammatory activities of hot water extract obtained from *Geranium thunbergii* using different extraction temperatures and times. Journal of the Korean Society of Food Science and Nutrition. 47: 1006 - 1013 (2018)

Surendraraj A, Farvin KHS, Anandan R. Antioxidant potential of water hyacinth (*Eichornia crassipes*): *In vitro* antioxidant activity and phenolic composition. Journal of Aquatic Food Product Technology. 22: 11 - 26 (2013)

Tana B, Choudhary B, Mishra A, Chauhan OP, Patel MK, Shokralla S, El-Abedin TZ, Elansary HO, Mahmoud EA. Antioxidant, scavenging,

reducing, and antio-proliferative activities of selected tropical brown seaweeds confirm the nutraceutical potential of *Spatoglossum asperum*. Foods. 10: 2482 (2021)

Tang Q, Huang K, Liu J, Wu S, Shen D, Dai P, Li C. Fine particulate matter from pig house induced immune response by activating TLR4/MAPK/NF-KB pathway and NLRP3 inflammasome in alveolar macrophages. Chemosphere. 236: 124373 (2019)

Vishchuk OS, Ermakova SP, Zvyagintseva TN. Sulfated polysaccharides from brown seaweeds *Saccharina japonica* and *Undaria pinnatifida*: Isolation, structural characteristics, and antitumor activity. Carbohydrate Research. 346: 2769 - 2776 (2011)

Wang SM, Yu DJ, Song KB. Physicochemical characteristics of kohlrabi slices dehydrated by the addition of maltodextrin. Journal of Food Science and Nutrition. 16: 189 - 193 (2011)

Waris G and Ahsan H. Reactive oxygen species: Role in the development of cancer and various chronic conditions. Journal of Carcinogenesis. 5 (2006)

Widyawati PS, Budianta TDW, Kusuma FA, Wijaya EL. Difference of solvent polarity to phytochemical content and antioxidant activitiy of *Pluchea indicia Less* leaves extracts. International Journal of Pharmacognosy and Phytochemical Research. 4: 850-855 (2014)

Xu X, Liu A, Hu S, Ares I, Martínez-Larrañaga MR, Wang X, Martínez M, Anadón A, Martínez MA. Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. Food Chemistry. 353: 129488 (2021)

Yeo JD and Shahidi F. Revisiting DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as a useful tool in antioxidant evaluation: A new IC_{100} concept to address its limitations. Journal of Food Bioactives. 7: 36 - 42 (2019)

Yi BR, Kim MJ, Lee JH. Lipid oxidation and antioxidant mechanisms in different matrix. Food Science and Industry. 51: 2 (2018)

Yi L, Ma S, Ren D. Phytochemistry and bioactivity of citrus flavonoids: a

focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. Phytochemistry Reviews. 16: 479 - 511 (2017)

Yin NS, Abdullah S, Phin CK. Phytochemical constituents from leaves of *Elaeis guineensis* and their antioxidant and antimicrobial activities. International Journal of Pharmacy and Pharmaceutical Sciences. 5: 137-140 (2013)

Zhang Y, Xu M, Þorkelsson G, Aðalbjörnssona BV. Comparative monosaccharide profiling for taxon differentiation: An example of Icelandic edible seaweeds. Biochemical Systematics and Ecology. 104: 104485 (2022)

Zhong H, Gao X, Cheng C, Liu C, Wang Q, Han X. The structural characteristics of seaweed polysaccharides and their application in gel drug delivery systems. Marine Drugs. 18: 12 (2020)

Zou Z, Xi W, Hu Y, Nie C, Zhou Z. Antioxidant activity of citrus fruits. Food Chemistry. 196: 885-896 (2016)

Zulaikhah ST. The role of antioxidant to prevent free radicals in the body. Sains Medika. 8: 39-45 (2017)

Sargassum horneri 에탄올 추출물의 분획 및 구조적 특성 분석

신 재 홍

제주대학교 대학원 식품공학과

요약

본 연구에서는 괭생이모자반 (*Sargassum horneri*)의 에탄올 추출물(SHE)과 잔기 (SHE-R) 및 용매 분획물 (S-SHE, T-SHE, P-SHE, PS-SHE)의 활성 화합물을 분석하고, 그들의 항산화 활성을 평가하였다. 총 폴리페놀 함량은 P-SHE에서 31.91 mg GAE/g, 총 플라보노이드 함량은 T-SHE에서 320.60 mg QE/g으로 높은 값을 나타내었다. Sulfate group contents는 SHE-R과 PS-SHE가 다른 분획물에 비해 높은 값을 나타내었고, 환원당은 SHE-R과 T-SHE에서 각각 0.01, 0.06%로 나타났다. 괭생이모자반 에탄올 추출물 및 분획물의 sterol 함량을 HPLC로 정량한 결과, fucosterol이 모든 시료에서 검출되었으며, n-hexane으로 분획한 S-SHE에서 25.96 mg/100 g으로 가장 높았다. 단순단당류 및 이당류를 분석한 결과, SHE에서 fucose, fructose, glucose, sucrose, maltose, 및 lactose가 검출되었으며, 모든 SHE 용매 분획물에서 fucose가 검출되었다. 총 11종의 phenolic acid를 분석한 결과 gallic acid가 S-SHE를 제외한 모든 추출물과 분획물에서 검출되었으며, T-SHE에서는 catechin이 P-SHE에서는 vanillic acid가 추가로 검출되었다. 또한 UPLC-Q-TOF-MS를 사용하여 P-SHE에서 phenolic acid를 분석한 결과 gallic acid가 P-SHE의 주요 페놀성 화합물임을 확인하였고, gallic acid esterified glucose를 동정하였다. 항산화 활성은 괭생이모자반 에탄올 추출물 및 분획물 중 P-SHE에서 전반적으로 가장 높은 활성을 보였다. 특히 금속 킬레이팅 결과는 대조구로 사용한 BHT (107.35%)와 유사한 값 (94.18%)을 나타내며 높은 금속 킬레이팅 능력을 보였다. 괭생이모자반 에탄올 추출물 및

분획물의 항산화제로서의 효능을 확인하기 위해 미세먼지를 첨가하여 산화를 유도한 후 항산화 활성을 조사하였다. 미세먼지의 농도를 0.125 mg/mL로 처리하였을 경우, 괭생이모자반 에탄올 추출물 및 분획물의 DPPH free radical, $ABTS^{+}$ radical 소거활성, hydrogen peroxide 소거활성, 금속 킬레이팅, 환원력 평가 결과가 감소하는 경향을 보였으나 괭생이모자반 에탄올 추출물 및 분획물이 미세먼지로부터 유도된 산화에서도 항산화 활성을 나타낸 것으로 판단된다. 따라서 괭생이모자반 추출물과 용매 분획물은 다양한 생리활성 물질을 포함하고 있으며 높은 항산화 활성을 보여주었으므로 특히 괭생이모자반 분획물은 항산화 기능성 식품소재로의 이용 가능성이 높을 것으로 기대된다.