Thesis for the Degree of Master of Science

In vitro Characterization of Bioactive Compounds Extracted from Sea Urchin (*Stomopneustes variolaris*) using Green and Conventional Techniques

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청정 및 기존의 기술을 이용하여 성게에서 (*Stomopneustes variolaris*) 추출한 생체활성 화합물의 *in vitro* 특성분석

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In vitro Characterization of Bioactive Compounds Extracted from Sea Urchin (*Stomopneustes variolaris*) using Green and Conventional Techniques*

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Abstract

Sea urchins belong to the phylum Echinodermata and are abundant in tropical and subtropical seas around the world and around 800 species are identified uptodate. The edible gonads of some sea urchins are called “uni” or “roe” across Southeast Asia and the Mediterranean countries. They are expensive luxury food with high nutritional content. But the gonads are a small portion of the sea urchin’s total body, whose inedible shells, spines, internal fluids, and organs are regularly discarded as waste. This high waste generation in urchin fishery and processing contributes to environmental issues, presenting a need for more productive utilization of these waste materials. Although an increasing amount of literature demonstrates potential antioxidant, antidiabetic, anti-inflammatory, and anticancer bioactivities of different compounds (mainly polyphenols, flavonoids, proteins, and polysaccharides) extracted from various body parts of different sea urchin species, most studies employed conventional organic solvent-assisted extraction methods. This study investigated the *in vitro* bioactivities of extracts obtained from viscera, spines, shells, and gonads of *Stomopneustes variolaris* using subcritical water extraction (SWE) at 110°C, 150°C, 190°C, and 230°C and Soxhlet extraction (SE). The highest amounts of phenolics (22.68±0.05 mg GAE/g), flavonoids (27.11±0.10 mg RE/g), and proteins (40.25±0.84 mg BSA/g) were recorded from gonads at 230°C, whereas

maximum sugar content (23.38±1.30 mg glucose/g) was in viscera at 150°C. Gonads at 230°C exhibited the highest DPPH activity (78.68±0.18 %), whereas viscera at 150°C exhibited the highest $ABTS^{+}$ (98.92±1.27%) and anti-inflammatory activity (37.13±9.94%). Viscera at 110°C claimed the highest amylase inhibition (42.46±0.83%), and spines at 150°C had the highest anticancer activity (IC_{50}=767.47 µg/mL). SWE achieved superior results in bioactive compound recovery and detected higher levels of bioactivities ($p<0.05$). The outcomes of the current study demonstrated that SWE was able to recover more bioactive compounds from sea urchin *S. variolaris* than SE, including total phenolics, flavonoids, proteins, and sugars. In addition, the *in vitro* antioxidant, antidiabetic, anti-inflammatory, and anticancer activities of SWE were higher than those of SE. However, the SWE of viscera and gonads showed higher bioactive compound recovery compared to shells and spines. Despite the yield of the bioactive compound, all extracts demonstrated considerable bioactivities. Therefore, SWE could be useful for the extraction of bioactive compounds from sea urchin S. variolaris for advanced applications. For example, it can be used in the food industry as an animal extract-based food additive or a nutrition supplement including phenolics, flavonoids, polisachcharaides and proteins. Also the antioxidant capacity of the SWE showed the potential appilaction in the cosmetic industry as an active ingredient. Antioxidant, antidiabetic, anti-inflammatory, and anticancer activities demostrated the potential use of these extracts in phamacutical industry so that develop thereputic treatements for diabetic, cancers and infalamatory diseases. Additionally, these extracts can be incoperated as an active ingredient in bioactive film formulation to produce active food packageing materials.

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1. Introduction

1.1. Sea urchins and their bioactive compounds

Sea urchins belong to the phylum Echinodermata and are abundant in tropical and subtropical seas around the world and around 800 species are identified uptodate. The edible gonads of some sea urchins are called “uni” or “roe” across Southeast Asia and the Mediterranean countries (Kuwahara et al., 2009). They are expensive luxury food with high nutritional content (Amarowicz, Synowiecki, & Shahidi, 2012; Kuwahara et al., 2009). Therefore, the global fishery of sea urchin has increased and South East Asian and pacific countries show a major contribution in global landing (**Fig. 1**). The gonads are a small portion (approximately 10%) of the sea urchin’s total body (Zhou et al., 2011), whose inedible shells, spines, internal fluids, and organs are regularly discarded as waste. This high waste generation in urchin fishery and processing contributes to environmental issues, presenting a need for a more productive utilization of these waste materials (Hou, Carne, McConnell, Mros, et al., 2020; Hou et al., 2016).

Stomopneustes variolaris is a highly abundant sea urchin species in Sri Lankan coastal waters and is common in the Indo-Pacific region (Gayashan & Jayakody, 2014). This edible sea urchin species is an underexploited resource in Sri Lanka and has the potential to develop as an industry in the near future (Jinadasa, De Zoysa, Jayasinghe, & Edirisinghe, 2016). Despite this, the bioactivities of this species have not been studied in depth.

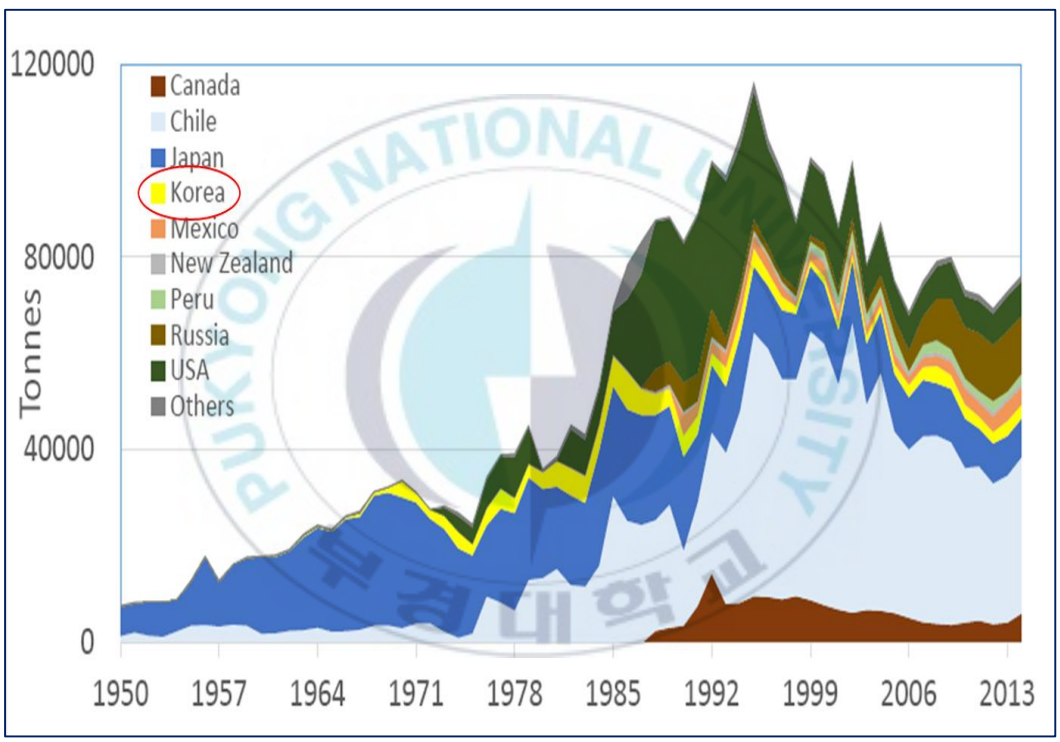

Fig. 1. Global landing of sea urchin (Source:Stefánsson et al. , 2017)

Currently, the food and pharmaceutical industries have more interest in natural bioactive compounds than in synthesized compounds as natural bioactive compounds have fewer adverse side effects. But the extraction method is a critical factor determining the quality and safety of the compounds.

Although an increasing amount of literature (**Table 1**) demonstrates potential antioxidant, antidiabetic, anti-inflammatory, and anticancer bioactivities of different compounds (mainly polyphenols, flavonoids, proteins, and polysaccharides) extracted from various body parts of different sea urchin species, most studies employed conventional organic solvent-assisted extraction methods (Archana & Babu, 2016; Brasseur et al., 2017; Haug et al., 2002; Hou, Carne, McConnell, Bekhit, et al., 2020; Hou, Carne, McConnell, Mros, et al., 2020; Kuwahara et al., 2009; Liu et al., 2007; Salem et al., 2017; Sheean et al., 2007; Soleimani, Moein, Yousefzadi, & Amrollahi Bioki, 2016).

Table 1. Some identified bioactivities of bioactive compounds extracted from different body parts of various sea urchin species using different extraction methods (Litreature review).

Sea Urchin Species	Body part	Extraction method/ solvent	Compound	Bioactivity	Study
Strongylocentrotus nudus	Eggs	Aqueous extraction followed by ethanol precipitation	polysaccharides	Antitumor activity	(Liu et al., 2007)
Heliocidaris erythrogramma	Gonads	Dichloromethane methanol and aqueous	NM	Antioxidant, Anti-inflammatory & anti-tumor activities	(Sheean et al., 2007)
Strongylocentrotus droebachiensis	Intestinal organs, eggs, gills, and body wall	Acetonitrile	NM	Antibacterial activity	(Haug et al., 2002)
Evechinus chloroticus	Shell and spine	Ethyl acetate, Chloroform & Diethyl ether	PHNQ	Anti-microbial Anti-inflammatory & Antioxidant activities	(Hou, Carne, McConnell, Bekhit, et al., 2020)
Evechinus chloroticus	Shell and spine	Macro porous resin	PHNQ	Anti-microbial Anti-inflammatory & Antioxidant activities	(Hou, Carne, McConnell, Mros, et al., 2020)
Paracentrotus lividus	Eggs	Papain digestion	Sulfated polysaccharides	Anti-inflammatory, Analgesic & Gastro protective activities	(Salem et al., 2017)
Echinometra mathaei, Diadema savignyi, Tripneustes gratilla & *Toxopneustes pileolus*	Tests and spines	Diethyl ether	Spinochromes	Antibacterial, antioxidant, inflammatory and cytotoxic activities	(Brasseur et al., 2017)
Echinometra mathaei	Spines, shells, gonads and Aristotle's lantern	n-hexane, ethyl acetate and methanol	Phenols and flavonoids	Antioxidant , α-amylase inhibition, & anti-inflammatory activities	(Soleimani, Moein, Yousefzadi, & Bioki, 2016)
Stomopneustes variolaris	Gonads	Methanol	Phenols	Antioxidant activity	(Archana & Babu, 2016)
Anthocidaris crassispina	Shells	Diethyl ether	PHNQ	Antioxidant activity	(Kuwahara et al., 2009)

NM: Not mentioned; PHNQ : Polyhydroxylated naphthoquinone.

1.2. Subcritical water extraction

Water at a temperature between 100°C and 374°C and pressure between 0.1 and 22 MPa defined as subcritical water (**Fig. 2**) (Cvjetko Bubalo, Vidović, Radojčić Redovniković, & Jokić, 2018) . Subcritical water extraction (SWE) uses water near its critical state, which is a temperature beyond the boiling point of water combined with a high enough pressure to keep water in the liquid state.

At a near-critical state, the dielectric constant and density of water decreases, and water diffusivity and solubility of organic compounds increases (**Fig. 3**). This makes water an efficient solvent for the extraction of bioactive compounds (Zhang, Wen, Zhang, Duan, & Ma, 2020).

As a green solvent that is non-toxic, non-flammable, and does not produce greenhouse gases and wastes, SWE is overall more green than conventional methods. Also, since water is readily available and a renewable resource, it can function as a more economical extraction solvent while also contributing to higher quality extracts compared to conventional solvent extraction. Though there are upfront costs to SWE systems and energy consumption is considerable, SWE is an economical, effective, and overall green technique of recovering nonpolar and polar bioactive compounds such as phenols, flavonoids, polysaccharides, and proteins in contrast to conventional extraction methods (Ho & Chun, 2019). However, prior research on sea urchin materials has not utilized SWE.

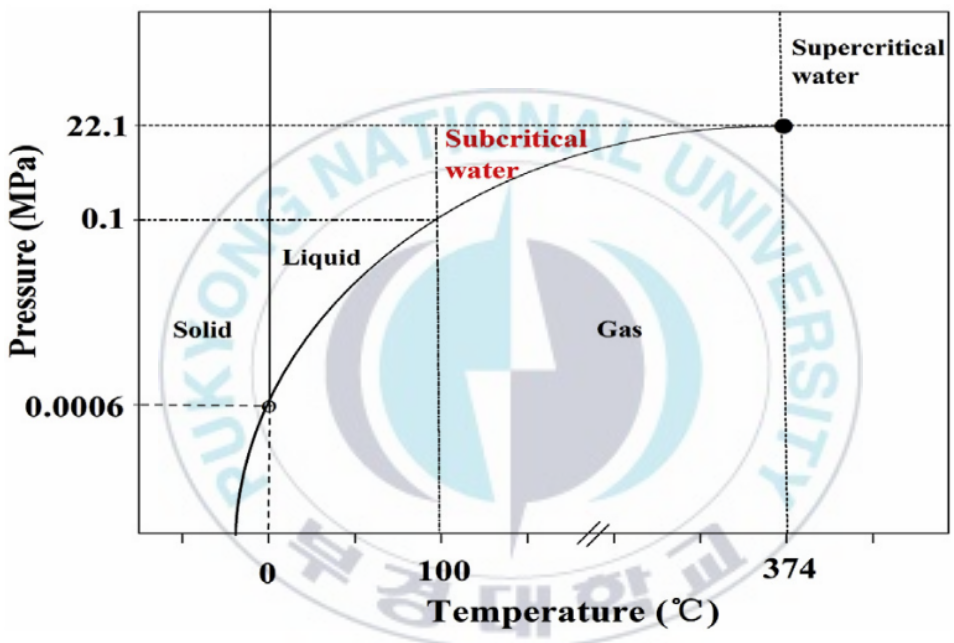

Fig. 2: Physical state of water at different temperature and pressure (source:Zhang et al., 2020)

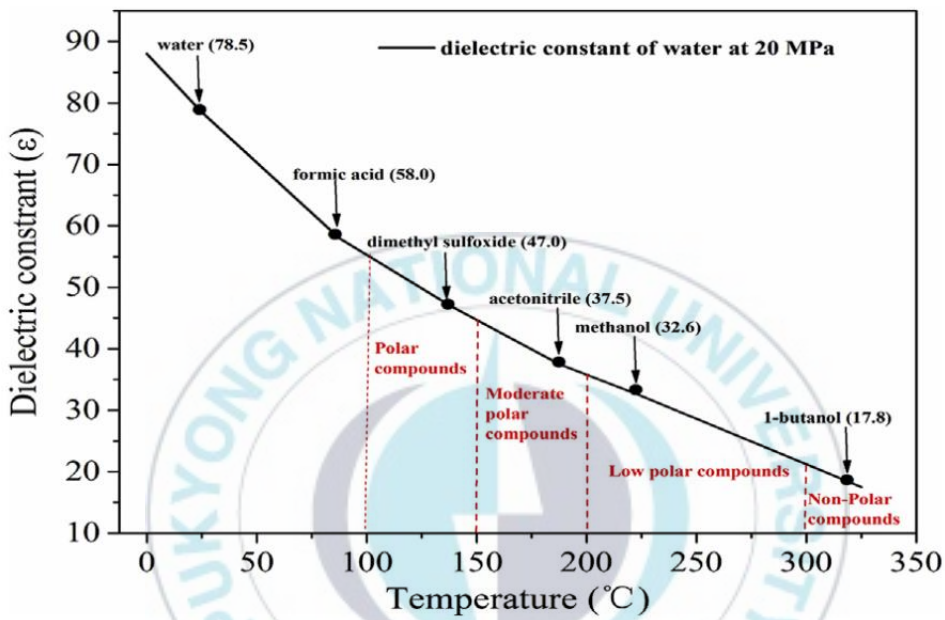

Fig. 3: Changes of water dielectric constant as a function of the temperature at constant pressure (20 MPa). The figure presents the dielectric constant values equivalents to some common organic solvents at room temperature and pres- sure (25 °C and 0.1 MPa). (source:Zhang et al., 2020)

1.3. Objectives of the study

Primary objective was to extract and characterize the bioactive compound from the different body parts of the sea urchin (*S. variolaris*) using SWE. Thus, this study used SWE to recover the bioactive compounds (such as phenols, flavonoids, polysaccharides, and proteins) of *S. variolaris*. SWE was used at four extraction temperatures (110°C, 150°C, 190°C, and 230°C), and Soxhlet extraction (SE) with 70% ethanol was used for comparison. The study examined four body parts. The bioactive potentialities of extracts from the viscera, spines, shells, and gonads were each measured using *in vitro* antioxidant, antidiabetic, anti-inflammatory, and anticancer assays.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, Gallic acid, Rutin, Bovine Serum Albumin (BSA), 2, 2-azinobis-3 ethyl benzothiazoline-6-sulphonic acid ($ABTS^{+}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and α-amylase, 5-Hydroxymethylfurfural (5-HMF), 5-methylfurfural (5-MF), chlorogenic acid, catechin, ferulic acid, and quercetin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MI, USA). All other reagents used in this study were of High-Performance Liquid Chromatography (HPLC) or analytical grade. Human umbilical vein endothelial cells (HUVEC), human normal embryonic kidney cells (HEK-293), and cervix adenocarcinoma cells (HeLa) were purchased from American Tissue Culture Collection (Manassas, VA, USA). Minimum Essential Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and antibiotic solution of penicillin/streptomycin were obtained from Mediatech Corning (Manassas, VA, USA).

2.2. Raw materials

Whole *S. variolaris* sea urchins were purchased from P.N. Fernando & Company Pvt. LTD., Ja-Ela, Sri Lanka, and imported to South Korea in frozen conditions.

2.3. Sample preparation

Frozen sea urchins were thawed to room temperature. Spines, shells, and gonads were separated and washed in a cold stream of water. The viscera (internal organs, body fluid, and Aristotle lantern) were collected separately. All of the samples (which had the moisture content of 15.75 ± 0.77%, 24.21 ± 3.82%, 68.88 ± 2.77%, and 67.80 ± 1.66% in the spines, shells, viscera, and gonads, respectively) were freeze-dried (HyperCOOl freeze drier, HC8080, Gyrozen Co., Ltd, Daejeon, Republic of Korea), crushed into powder using a mechanical grinder (2500Y-Dong Yi multifunctional grinder, Yongkang Boou Hardware Products Co., Ltd. China), and sieved with a stainless-steel sieve (with a mesh size of 500 μm). Then, the powdered samples were placed in air-tight bottles and stored in the dark at −20°C until the extractions. Sample preparation process is showed in **Fig. 4.**

Fig. 4: Sample preparation process. (a) imported sample, (b) thawed sample, (c) dissection of the samples, (d) separation of the body parts, (e) freeze drying of the samples, (f) grinding the dried samples into pawder.

2.4. Extraction methods

Soxhlet extraction and Subcritical water extraction were done according to the methods described by Ho & Chun (2019) with slight modifications.

2.4.1. Soxhelet extraction

Four grams of the powder of each sample was extracted separately with 140 mL of 70% ethanol for 7 hours in an Soxhlet apparatus (**Fig. 5**). The extracts were then concentrated by evaporating them at 40°C in a rotary evaporator until the final volume of 22 ± 2 mL. The concentrated samples (**Fig. 6**) were stored in the dark at 4°C until further analysis.

Fig.5: Soxhlet extraction system

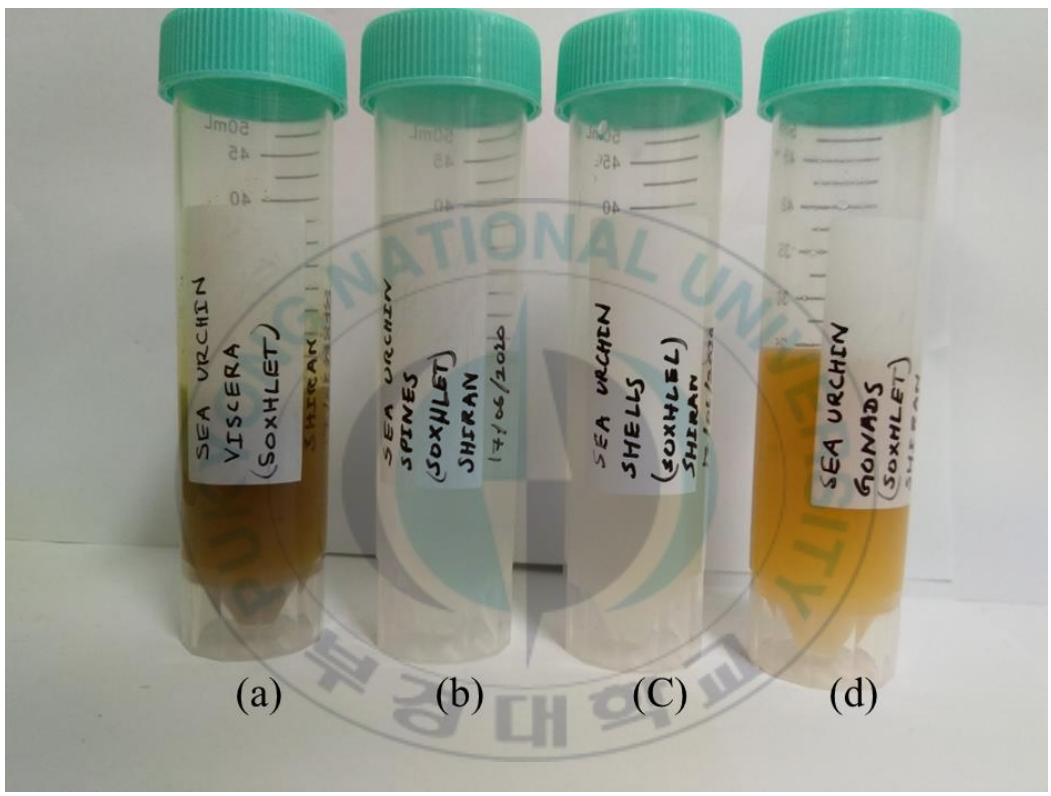

Fig. 6: Extracts obtained from the soxhlet extraxtion (a) viscera; (b) spines; (c) shells; (d) gonads

2.4.2. Subcritical water extraction

A small-scale SWE apparatus (**Fig. 7**) was used for the extraction process. Each of the four samples were separately extracted in four different temperatures: 110°C, 150°C, 190°C, and 230°C. A 200 mL volume reactor was filled with 150 mL of distilled water mixed with 4g of the powdered sample. Then, the reactor was sealed and filled with nitrogen gas. Next, the rotator was switched on, followed by the electric heater. After the reactor reached the target temperature, 15 min of extraction time began. A solid: liquid ratio of 1:37.5, an extraction time of 15 min, and pressure of 50 bars were kept constant during the extractions. After completion of each extraction, the safety valve was opened slowly to let the extract cool down at the cooler, after which the extract was collected from the collector. The collected extract was centrifuged at 7,900g in 4°C for 20 min and filtered under a slight vacuum through a Buchi vacuum pump V100 with an F1113 grade filter paper. 15 mL aliquots of filtered extracts (**Fig. 8**) were transferred into plastic tubes and stored at 4°C in the dark until further analysis. The rest of the extracts were freeze-dried (**Fig. 9**) and stored in dark at 4°C until analysis.

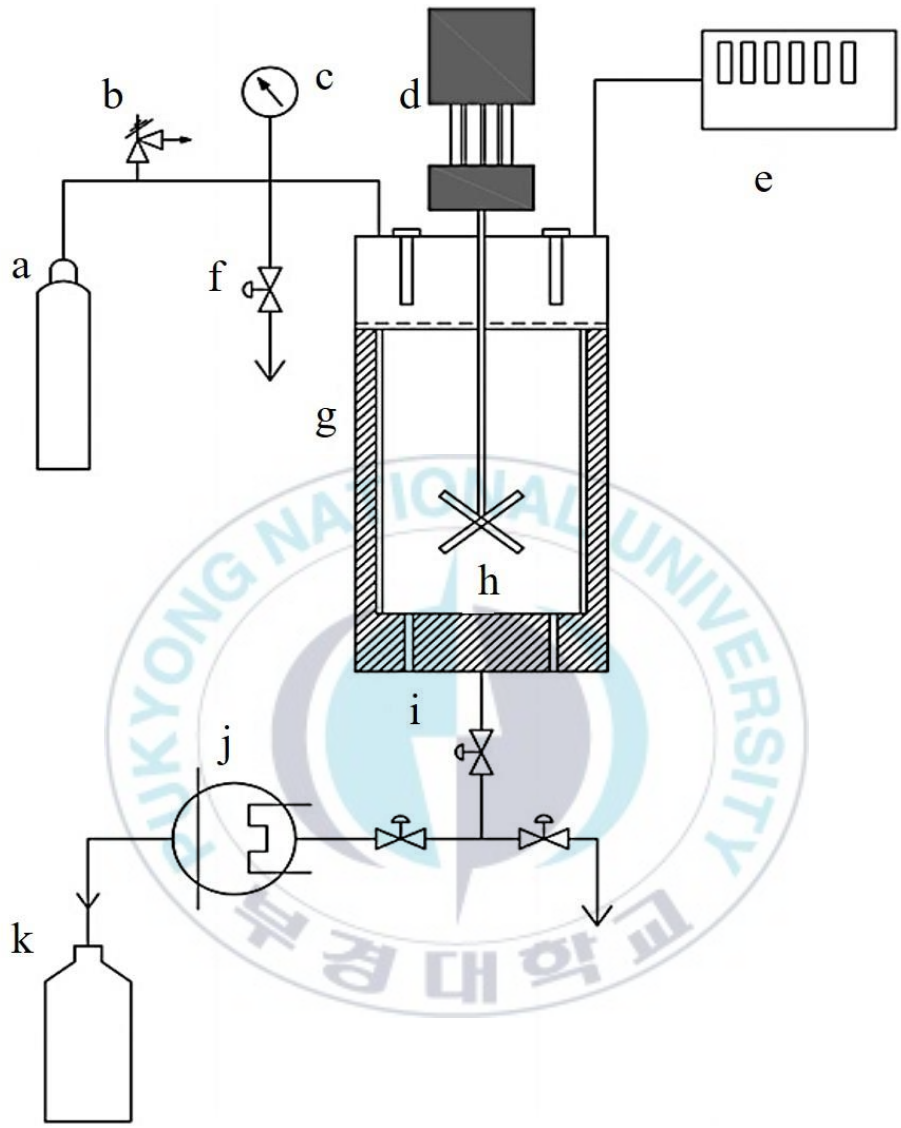

Fig. 7. Flow diagram of subcritical water experimental apparatus (modified from: Ho & Chun, 2019). (a) Nitrogen gas; (b) Safety valve; (c) Pressure gauge; (d) Rotator; (e) Controller of temperature and stirring speed; (f) Control valve; (g) High pressure reactor; (h) Impeller; (i) Electric Heater; (j) Cooler; (k) Collector.

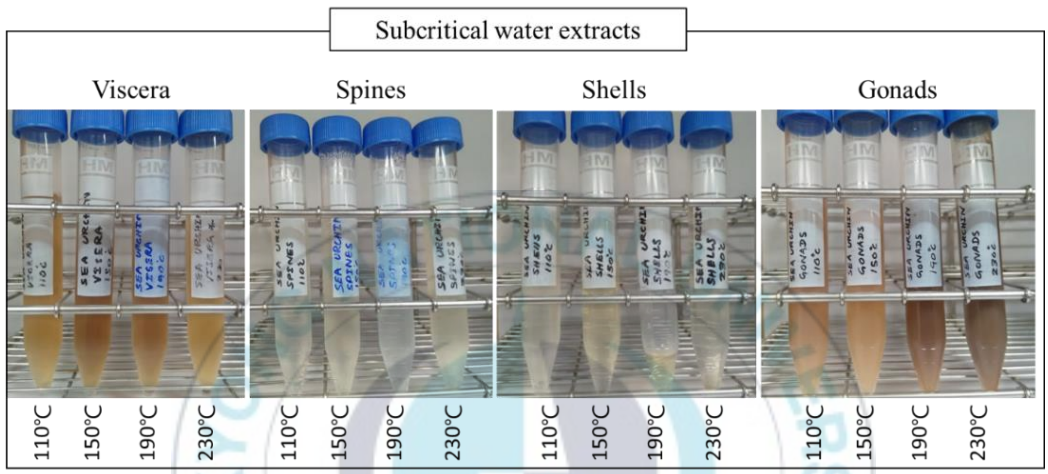

Fig. 8: Extracts obtained from the subcritical water extraction

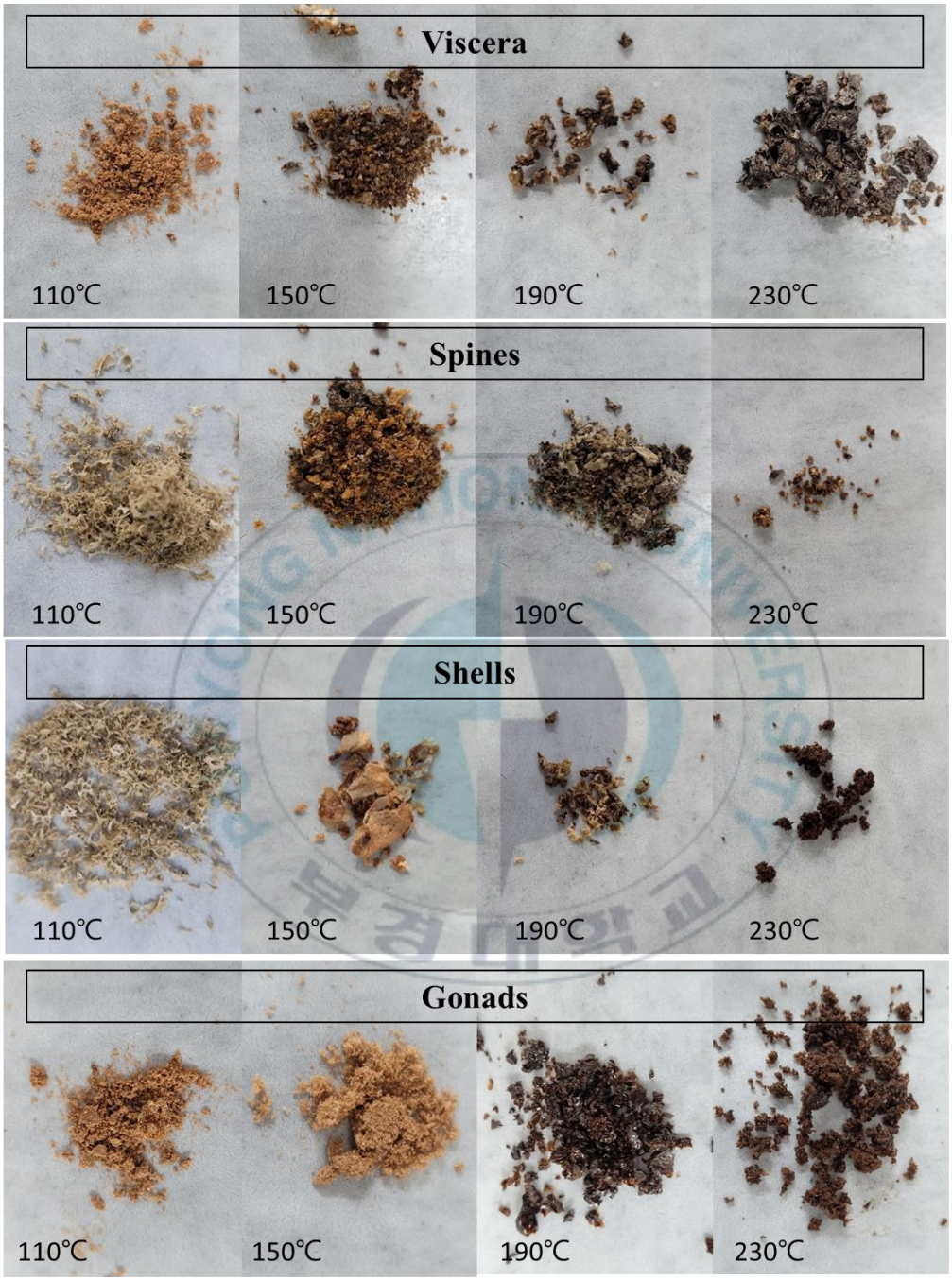

Fig. 9: Powders obtained after freeze drying the subcritical water extracs

2.5. Chemical analysis

2.5.1. Analysis of total phenolic content (TPC)

TPC was assessed with reference to Ho & Chun (2019) with modifications. 0.5 mL of the sample was mixed with 2.5 mL of a 0.2 N Folin–Ciocalteu reagent and allowed to stand for 5 min. Then, 2 mL of 75 g/L sodium carbonate (Na_2CO_3) was added to the mixture. The absorbance of the reaction mixture was measured at 760 nm after a 2 h incubation period at room temperature. Gallic acid was used as the standard, and the TPC was expressed as a milligram gallic acid equivalents per gram of a dried sample (mg GAE/g).

2.5.2. Analysis of total flavonoid content (TFC)

TFC was measured using the method adopted from Ho & Chun (2019) along with modifications. One milliliter of the sample was diluted with 2 mL of distilled water. Then, 0.15 mL of 5% $NaNO_2$ was added and left to stand for 6 min. Next, 0.15 mL of 10% $AlCl_3$ was added and again allowed to stand for 6 min. Two milliliters of 4% NaOH was added into the mixture, vortexed, and allowed to stand for 15 min, followed by measurement of the absorbance of the reaction mixture at 510 nm. Rutin was used as the standard, and TFC was expressed as a milligram rutin equivalents per gram dried sample (mg RE/g).

2.5.3. Analysis of total protein content (TPrC)

Pomory assay (Pomory, 2008) was employed to analyze the TPrC. A fresh solution was prepared by mixing 1% $CuSO_4.5H_2O$, 2% tartrate, and 2% Na_2CO_3 at a ratio of 1:1:100. Five milliliters of that fresh solution were mixed with 0.5 mL of the sample, vortexed for a few seconds, and allowed to stand for 10 min. Then, 0.5 mL of a 1 N Folin–Ciocalteu reagent was added and vortexed. The mixture was allowed to stand for 2 h, and absorbance was then measured at 660 nm. BSA was used as a standard, and TPrC was expressed as milligrams of BSA equivalents per gram of a dried sample (mg BSA/g).

2.5.4. Analysis of total sugar content (TSC)

TSC was analyzed according to the method reported by Ho & Chun (2019) with slight modifications. 0.5 mL of the sample was mixed with 1 mL of a phenol solution (2% w/w), followed by rapid addition of 2.5 mL of concentrated sulfuric acid (H_2SO_4). The mixture was kept for 10 min in the dark for color development. Then, it was allowed 30 min of cooling in a water bath at 22°C, and absorbance was measured at 490 nm. The TSC was expressed as milligram glucose equivalents per gram dried sample (mg glucose/g) referenced to the standard sugar mixture (glucose, fructose, and galactose; 1:1:1) (Sigma-Aldrich).

2.6. Assessment of bioactivity

2.6.1. Analysis of the antioxidant activity

The following two assays were performed according to Roy, Getachew, Cho, Park, & Chun (2020) with minor modifications to evaluate the antioxidant activity.

2.6.1.1. DPPH radical scavenging activity

250 microliters of the sample were mixed with 3.75 mL of a 0.2 mM DPPH ethanolic solution and incubated in the dark for 30 min. Then, the absorbance was measured at a 517 nm reference with a blank. Trolox (300 µg/mL dissolved in ethanol) was used as a standard reference. The percentage of DPPH radical scavenging activity was measured according to the following equation:

$$DPPH\ \ Radical\ \ Scavenging\ \ Activity\ \ (\%) = \left[1 - \left(\frac{A_s - A_b}{A_c}\right)\right] \times 100,$$

where A_s - absorbance of the mixture of sample and the DPPH solution, A_b - absorbance of the blank that contains only the sample, and A_c - absorbance of the control that contains only the DPPH solution.

2.6.1.2. $ABTS^+$ radical scavenging activity

Equal volumes of 7 mM $ABTS^+$ and 2.45 mM potassium persulfate were mixed and allowed to stand in the dark at room temperature for 16 h to prepare the stock solution. Then the $ABTS^+$ stock solution was diluted with 94% ethanol until the absorbance of 0.70 ± 0.02 at 734 nm to prepare the working solution. 2.75 ml of diluted $ABTS^+$ was mixed with 250 µL of the sample. After 6 min incubation in the dark, the absorbance at 734 nm was measured against a blank. Trolox (300µg/mL dissolved in ethanol) was used as a standard reference. The following equation was used to calculate the percentage of radical scavenging activity of $ABTS^+$:

$$ABTS^+ \ Radical \ Scavenging \ Activity \ (\%) = \left[1 - \left(\frac{A_s - A_b}{A_c}\right)\right] \times 100,$$

where A_s - absorbance of the mixture of sample and the $ABTS^+$ solution, A_b - absorbance of the blank that contains only the sample, and A_c - absorbance of the control that contains only the $ABTS^+$ solution.

2.6.2. Analysis of *in vitro* antidiabetic activity

The *in vitro* antidiabetic activity was measured using an α-amylase inhibitory assay according to Kwon, Apostolidis, & Shetty (2008) with modifications. A total of 500 µL of the α-amylase solution (0.5 mg/mL) in a 0.02 M sodium phosphate buffer (pH 6.9 with

0.006 M sodium chloride) was added to 500 µL of the sample and incubated for 10 min at 25°C. Then, 500 µL of a 1% starch solution in a 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was introduced to the mixture and incubated at 25°C for 10 min. The reaction was stopped with the addition of 1.0 mL of dinitrosalicylic acid color reagent, and the test tubes were kept in a boiling water bath for 5 min. Then, they were let to cool to room temperature, and the reaction mixture was diluted by adding 10 mL of distilled water. Absorbance (A) was measured at 540 nm, and α-amylase inhibition percentage was presented relative to a blank control according to the following equation:

$$Inhibition(\%) = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}}\right] \times 100.$$

2.6.3. Analysis of *in vitro* anti-inflammatory activity

The *in vitro* anti-inflammatory activity was assessed according to the protein denaturation inhibition assay with slight modifications to the method described by Chandra, Chatterjee, Dey, & Bhattacharya (2012). A total of 500 µL of the sample was introduced to 2.5 mL of a fresh egg yolk albumin solution (6.6% v/v in PBS, pH 6.4). Distilled water was used for the control, and 500 µg/mL aspirin was used as a standard. Mixtures were incubated for 15 min at 37°C. Then, the samples were heated at 70°C for 5 min and allowed to cool to room temperature. Afterwards, the absorbance (A) was measured at 660 nm, and percentage inhibition of protein denaturation was calculated using the following equation:

$$Inhibition(\%) = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}}\right] \times 100.$$

2.6.4. Analysis of *in vitro* anticancer activity

In vitro HUVEC, HEK-293, and HeLa cell viability assays were employed according to the method described by Niyonizigiye et al. (2020) to assess the anticancer properties of the extracts. The powder obtained after freeze-drying of SWE was used to prepare different concentrations for the cell viability experiment to determine the 50% inhibition concentration (IC_{50}).

2.7. HPLC analysis of phenolic compositions

HPLC analysis was conducted according to the method described by Belguidoum, Amira-Guebailia, Boulmokh, & Houache (2014). The system included a Lunar® C18(2) 100A column (250 × 4.6 mm, 5 µm) with a UV-Vis detector set at 280 nm. The mobile phase contained 1% phosphoric acid in water (solvent A) and 1% phosphoric acid in acetonitrile (solvent B). The flow rate was maintained at 1 mL/min and the injection volume was 20 µL. Gallic acid, 5-HMF, 5-MF, chlorogenic acid, catechin, ferulic acid, and quercetin were used as standards. Solutions of each standard, concentration from 10 to 1000 µg/mL were prepared and used for the validation of linearity.

2.8. Statistical analysis

Data were analyzed using a one-way analysis of variance test along with Tukey's Post Hoc test at a 95% confidence level using SPSS software (version 18.00, SPSS Inc., IL, USA).

3. Results and discussion

3.1. Total flavonoid and phenolic content

TPC and TFC of the different extracts are presented in **Table 2**. SWE of gonads at 230°C were the highest TPC of all the samples, whereas SWE of spines at 110°C and SE of spines had the lowest TPC. When the SWE of different body parts of sea urchins was considered, the TPC exhibited variations with the extraction temperature. SWE of viscera at 150°C and 190°C had peak TPC, whereas 150°C, 190°C, and 230°C extracts gave peaks for the spines, shells, and gonads, respectively. All of the SWE of different body parts at 110°C indicated the lowest TPC. SWE of gonads, viscera, and shells had significantly higher TPC in contrast to the SE of similar body parts. But only the SWE of spines at 150°C and 190°C showed significantly higher TPC than that of their SE counterparts.

Methanolic extracts from the gonads, spines, and shells of purple sea urchin (*Echinometra mathaei*) reported phenolic contents of 0.0044, 0.3256, and 0.1781 mg GAE/g for the dry material, respectively (Soleimani, Moein, Yousefzadi, & Bioki, 2016). TPC values of SWE reported in the current study were higher than the TPC values reported by Soleimani et al. (2016), whereas SE indicated the opposite. Powell, Hughes, Kelly, Conner, & McDougall (2014) reported TPC of diethyl ether extracts from the sea urchin *Psammechinus miliaris* shells with 0.7–1.0 mg GAE/g dry weight material. However, extracts from the shells and spines of the *Evechinus chloroticus* using ethyl acetate, chloroform, diethyl ether (Hou, Carne, McConnell, Bekhit, et al., 2020), and different macroporous resins (Hou, Carne,

McConnell, Mros, et al., 2020) reported much higher TPC values than those in the current study. This may be due to the demineralization of shells and spines with strong acids such as hydrochloric or sulphuric acid before their extraction processes.

Table 2: Analysis of bioactive compounds from various extracts

Sample			Total Phenolic (mg GAE /g) Mean ± SD	Total Flavonoids (mg RE /g) Mean ± SD	Total Protein (mg BSA /g) Mean ± SD	Total Sugar (mg glucose /g) Mean ± SD
Extraction Method	Body part	Extraction Temperature				
SWE	Viscera	110ºC	4.13 ± 0.02 ^{g}	1.59 ± 0.09 ^{h}	2.66 ± 0.46 ^{h}	14.77 ± 0.17 ^{c}
		150ºC	9.38 ± 0.15 ^{d}	3.33 ± 0.02 ^{e}	19.68 ± 0.56 ^{d}	23.38 ± 1.30 ^{a}
		190ºC	9.44 ± 0.01 ^{d}	2.62 ± 0.10 ^{f}	17.43 ± 0.27 ^{e}	13.18 ± 0.29 ^{d}
		230ºC	6.96 ± 0.03 ^{e}	1.94 ± 0.04 ^{g}	4.06 ± 0.05 ^{g}	5.29 ± 0.11 ^{f}
	Spines	110ºC	0.05 ± 0.01 ^{m}	ND	ND	2.39 ± 0.08 ^{g,h}
		150ºC	1.04 ± 0.02 ^{i}	0.3 ± 0.02 ^{j}	ND	4.76 ± 0.35 ^{f}
		190ºC	0.24 ± 0.02 ^{l,m}	ND	ND	2.53 ± 0.25 ^{g,h}
		230ºC	0.98 ± 0.01 ^{i}	0.21 ± 0.03 ^{j,k}	ND	3.24 ± 0.21 ^{g,h}
	Shells	110ºC	0.43 ± 0.00 ^{l}	0.02 ± 0.02 ^{k}	ND	3.22 ± 0.08 ^{g,h}
		150ºC	1.98 ± 0.02 ^{h}	0.59 ± 0.00 ^{i}	ND	5.74 ± 0.19 ^{f}
		190ºC	4.28 ± 0.03 ^{g}	1.80 ± 0.22 ^{g,h}	3.25 ± 0.35 ^{g}	14.59 ± 0.55 ^{c}
		230ºC	0.89 ± 0.04 ^{i,j}	0.11 ± 0.03 ^{j,k}	ND	3.35 ± 0.16 ^{g}
	Gonads	110ºC	6.76 ± 0.01 ^{f}	7.58 ± 0.10 ^{c}	15.62 ± 0.12 ^{f}	23.42 ± 0.28 ^{a}
		150ºC	10.34 ± 0.04 ^{c}	8.10 ± 0.09 ^{b}	26.07 ± 0.49 ^{c}	19.27 ± 0.35 ^{b}
		190ºC	18.87 ± 0.21 ^{b}	8.13 ± 0.08 ^{b}	38.78 ± 0.46 ^{b}	23.42 ± 0.50 ^{a}
		230ºC	22.68 ± 0.05 ^{a}	27.11 ± 0.10 ^{a}	40.25 ± 0.84 ^{a}	9.98 ± 0.29 ^{e}
SE	Viscera	85ºC	0.73 ± 0.00 ^{j,k}	1.97 ± 0.04 ^{g}	0.16 ± 0.02 ^{i}	2.06 ± 0.03 ^{h}
	Spines	85ºC	0.05 ± 0.00 ^{m}	0.15 ± 0.00 ^{j,k}	ND	0.40 ± 0.02 ^{i}
	Shells	85ºC	0.06 ± 0.02 ^{m}	0.33 ± 0.01 ^{j}	ND	0.52 ± 0.01 ^{i}
	Gonads	85ºC	0.66 ± 0.07 ^{k}	6.25 ± 0.03 ^{d}	0.31 ± 0.14 ^{i}	3.33 ± 0.10 ^{g}

SWE: Subcritical water extraction; SE: Soxhlet extraction; ND: Not detected; Each value is presented as mean ± SD (n = 3); Different superscripts in the same column indicate statistical differences ($P < 0.05$).

All SWE at 110°C had the lowest TFC, besides SWE of viscera, shells, and gonads that gave peak TFC at 150°C, 190°C, and 230°C, respectively. Visceral extracts from SWE at 150°C and 190°C, shells at 190°C, and gonads at all four temperatures demonstrated significantly higher TFC than those from corresponding SE. Soleimani et al. (2016) reported TFC (mg butylated hydroxyl toluene /g of dry material) in the ethyl acetate extracts of the purple sea urchin (*E. mathaei*) gonads (3.9 ± 0.41), shells (0.47 ± 0.27), and spines (4.81 ± 0.32). In the current study, SWE and SE of gonads were higher than Soleimani et al.'s values. In contrast, SWE and SE of spines found lower values. The SWE of shells, except at 110°C, resulted in higher TFC than that of Soleimani et al. (2016), whereas the SE of shells resulted in lower TFC. The better results from SWE may indicate its strong potential. During the SWE, the dielectric constant of water decreases, and hydrogen bonding weakens. Also, the density and polarity of water decrease. This enables the rate of phenolics and flavonoids dissolution (Zhang et al., 2020). At each extraction temperature, TPC in gonadal SWE significantly increased as temperature increased. In contrast, for both viscera and shells, TPC increased as temperature increased only up to 190°C. TPC values decreased at 230°C for both. Similarly, TFC increased from 110°C to 150°C but then decreased at higher temperatures. Flavonoids were not detected in SWE of spines at 110°C and 190°C. Overall, elevated temperature enhanced the breaking of chemical bonds, mass transfer rates, and molecular diffusion that facilitate the solubility of phenolic and flavonoid compounds (Lachos-Perez et al., 2018). Therefore, the temperature was one of the key factors for the extraction of such compounds in SWE (Ho & Chun,

2019). However, further elevation of temperature may cause the degradation of those compounds (Singh & Saldaña, 2011).

3.2. The total protein content

Protein content in percent of dry weight in different body parts of sea urchin *S. variolaris* was reported in the previous study as 36–51% in gonads,1–1.5% in spines, 2–6% in the body wall, 40–50% in the gut, 6–8% in gut content and 3–5% in Aristotle's lantern (Giese, Krishnaswamy, Vasu, & Lawrence, 1964). Therefore, TPrCs in extracts were analyzed and presented in Table 1. In the current study gut, gut content, body fluid, and Aristotle's lantern of sea urchin *S. variolaris* were collectively considered to be viscera.

Noticeable TPrCs were recorded from the SWE of gonads and viscera. Protein yield in SWE of gonads increased with the elevation of temperature. The increase of protein yields as the temperature increased in SWE of plant materials was demonstrated in previous studies (Ho & Chun, 2019; Sereewatthanawut et al., 2008). However, in the context of the SWE of viscera, the peak was at 150°C and then decreased. The lowest TPrCs were recorded in SE of viscera and gonads. This may be due to the low solubility of the protein in 70% ethanol (Ho & Chun, 2019; Pace et al., 2004). Proteins were not detected in the SWE and SE of spines and shells except for the SWE of shells at 190°C. Both the spines and body wall of sea urchins have a small amount of protein as they are largely made up of calcareous material (Giese et al., 1964).

3.3. The total sugar content

Polysaccharides have a variety of biological activities (Zhang, Wen, Zhang, Duan, & Ma, 2020). According to Liu et al. (2007) and Salem et al. (2017) sea urchin gonads, shells and spines are a potential source for polysaccharides. As SWE is a well-established method of extracting polysaccharides, the total sugar content of the extracts was analyzed. The highest TSC was recorded from SWE of gonads at 110°C and 190°C. Peak TSC was recorded from SWE of viscera at 150°C, spines at 150°C, and shells at 190°C. However, SWE of different body parts did not show a clear relationship between TSC and extraction temperatures.

Further, in the current study TSC values of all SWE from gonads were higher than the crude polysaccharide yield (5.6 mg/g of sample) of hot water (90°C) extraction from the gonads of the sea urchin (*Strongylocentrotus nudus*) reported in a previous study (Liu et al., 2007). At the near-critical state with elevated temperature, the dielectric constant of water reduces, which leads to the solubility power of water becoming similar to organic solvents. This can dissolve various medium and low polarity compounds. Therefore, SWE can result in more polysaccharides than hot water extraction.

Also, Salem et al. (2017) recorded 41.8% of TSC yield from the gonads of the sea urchin *Paracentrotus lividus* using papain digestion. That TSC value (46.8 mg/g of sample) was higher than the TSC values obtained in the current study. Salem et al's study indicated that *P. lividus* eggs are a good source of sulfated polysaccharides and neutral sugars including arabinose, galactose, glucose, and fucose. However, Jiao et al. (2015) showed polysaccharide content varies by sea urchin species and that the monosaccharide composition between sea urchin shells and gonads is similar.

3.4. Antioxidant activity

There is a growing interest in antioxidants, which prevents the toxic effects of free radicals in the human body as well as in food products (Willcox, Ash, & Catignani, 2004). Therefore, natural antioxidants are a large alternative source that has significant potential for reducing the dependence on chemically synthesized compounds (Ho & Chun, 2019).

Several previous studies have researched the antioxidant properties in extracts obtained from different body parts of various sea urchin species (Archana & Babu, 2016; Brasseur et al., 2017; Hou, Carne, McConnell, Bekhit, et al., 2020; Hou, Carne, McConnell, Mros, et al., 2020; Kuwahara et al., 2009; Sheean et al., 2007; Soleimani, Moein, Yousefzadi, & Amrollahi Bioki, 2016). A DPPH assay was the most employed antioxidant assay among these studies. Some of the previous reports indicated dose-dependent higher DPPH scavenging activity of urchin extracts than that in some standards, such as α-tocopherol (Kuwahara et al., 2009), BHT (Soleimani, Yousefzadi, Moein, Rezadoost, & Bioki, 2016), and Trolox (Brasseur et al., 2017).

Percentages of radical scavenging activities resulting from $ABTS^{+}$ and DPPH assays are presented in **Table 3**. SWE of viscera and gonads demonstrated higher antioxidant capacities than others. The highest activity for the $ABTS^{+}$ assay was reported from SWE of viscera at 150°C, and the lowest was from the SWE of spines at 190°C. The highest DPPH scavenging activity was recorded from the SWE of gonads at 190°C, and the lowest was from a shell extract at 190°C. All the SWE of spines and shells, except shells at 190°C, did not detect radical scavenging activity in the DPPH assay. Every SE showed the same lack of activity.

Table 3. Characterization of bioactivities of various extracts

Sample			ABTS^{+} (%) Mean ± SD	DPPH (%) Mean ± SD	Amylase inhibition (%) Mean ± SD	Protein denaturation inhibition (%) Mean ± SD
Extraction Method	Body part	Extraction Temperature				
Subcritical Water	Viscera	110ºC	69.89 ± 0.43 ^{g}	19.75 ± 5.18 ^{d,e}	42.46 ± 0.83 ^{a}	22.89 ± 1.73 ^{a,b,c,d}
		150ºC	98.92 ± 1.27 ^{a}	68.84 ± 7.91 ^{b}	38.71 ± 1.15 ^{a,b}	37.13 ± 9.94 ^{a}
		190ºC	89.08 ± 0.11 ^{b,c}	42.27 ± 0.69 ^{c}	39.63 ± 0.82 ^{a,b}	13.43 ± 0.31 ^{c,d,e,f}
		230ºC	86.86 ± 0.15 ^{c,d}	23.19 ± 0.84 ^{d}	32.41 ± 0.21 ^{c,d}	3.51 ± 2.54 ^{e,f}
	Spines	110ºC	18.27 ± 2.91 ^{m,n}	ND	19.41 ± 0.51^{h}	5.89 ± 6.11 ^{e,f}
		150ºC	37.46 ± 0.53 ^{j}	ND	41.85 ± 0.30 ^{a}	16.41 ± 3.32 ^{b,c,e}
		190ºC	15.20 ± 0.57 ^{n}	ND	28.70 ± 1.64 ^{d,e,f}	2.82 ± 1.36 ^{e,f}
		230ºC	29.20 ± 1.06 ^{k}	ND	29.93 ± 1.15 ^{d,e}	3.63 ± 1.30 ^{e,f}
	Shells	110ºC	23.12 ± 0.67 ^{l}	ND	32.01 ± 0.83 ^{g}	3.35 ± 0.14 ^{e,f}
		150ºC	49.37 ± 0.61 ^{i}	ND	32.89 ± 5.03 ^{c,d}	3.16 ± 0.40 ^{e,f}
		190ºC	70.80 ± 0.57 ^{g}	2.11± 0.28 ^{f}	22.37 ± 1.62 ^{g,h}	31.81 ± 1.06 ^{a}
		230ºC	18.90 ± 0.88 ^{m}	ND	27.92 ± 1.04 ^{d,e,f}	23.42 ± 8.28 ^{a,b,c,d}
	Gonads	110ºC	80.02 ± 1.61 ^{f}	62.20 ± 3.02 ^{b}	31.73 ± 0.66 ^{c,d,e}	15.53 ± 0.39 ^{d,e}
		150ºC	81.98 ± 0.61 ^{e,f}	13.71 ± 1.29 ^{e}	23.29 ± 1.24 ^{f,g,h}	9.14 ± 3.41 ^{d,e,f}
		190ºC	91.29 ± 0.81 ^{b}	82.73 ± 0.58 ^{a}	26.80 ± 0.48 ^{e,f,g}	29.23 ± 0.07 ^{a,b,c}
		230ºC	89.87 ± 0.20 ^{b,c}	78.68 ± 0.18 ^{a}	24.17 ± 0.56 ^{f,g,h}	29.81 ± 7.93 ^{a,b,c}
Soxhelt	Viscera	85ºC	83.88 ± 0.66 ^{d,e}	ND	32.99 ± 0.18 ^{c,d}	ND
	Spines	85ºC	34.73 ± 1.03 ^{j}	ND	30.51 ± 0.16 ^{d,e}	ND
	Shells	85ºC	35.24 ± 1.84 ^{j}	ND	36.23 ± 4.39 ^{b,c}	3.95 ± 1.93 ^{e,f}
	Gonads	85ºC	63.46 ± 0.48 ^{h}	ND	8.75 ± 2.20 ^{i}	30.12 ± 3.88 ^{a,b}

ND: Not detected; Each value is presented as mean ± SD (n = 3); Different superscripts in the same column indicate statistical differences ($P < 0.05$).

Methanol extracts from the gonads of sea urchin *S. variolaris* showed similar antioxidant capacity with SWE of gonads (Archana & Babu, 2016). Enzymatic hydrolysates from purple sea urchin (*S. nudus*) gonads reported similar antioxidant activity in DPPH assay and suggested a positive correlation of protein content with antioxidant activity (Qin et al., 2011). The current study also demonstrated a positive correlation of TPrC with radical scavenging activity in the DPPH assay ($R^2 = 0.77$, $p < 0.05$) (**Fig.10**).

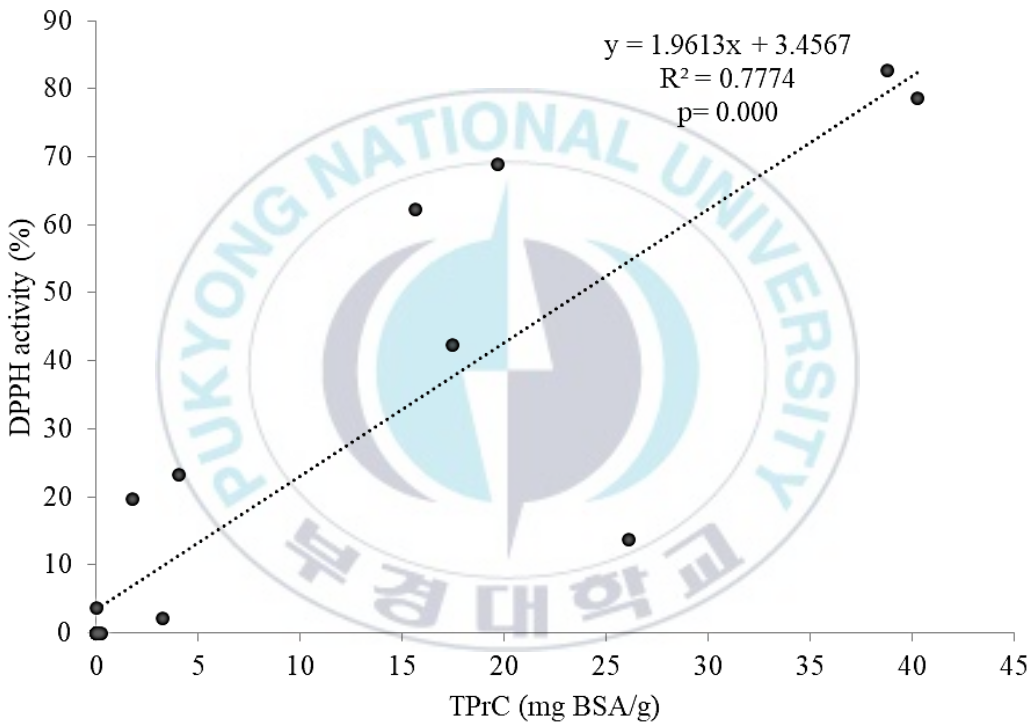

Fig. 10: Correlation of DPPH scavenging activities with TPrC

Polyphenols and flavonoids are renowned classes of compounds with demonstrated antioxidant capacity (Scalbert, Johnson, & Saltmarsh, 2005). Most of the studies related to the antioxidant activity of urchins' spines and shells targeted the polyhydroxynaphthoquinone (PHNQ) pigments (Hou, Carne, McConnell, Bekhit, et al., 2020; Hou, Carne, McConnell, Mros, et al., 2020; Kuwahara et al., 2009; Powell et al., 2014), which are considered to be a polyphenol compound (Powell et al., 2014). PHNQ pigments were also reported from the celomic fluid of sea urchins (Vasileva, Mishchenko, & Fedoreyev, 2017). Though the current study did not study PHNQ contents in extracts, it demonstrated a positive correlation of DPPH scavenging activities with TPC ($R^2 = 0.78$, $p < 0.05$) (**Fig. 11**).

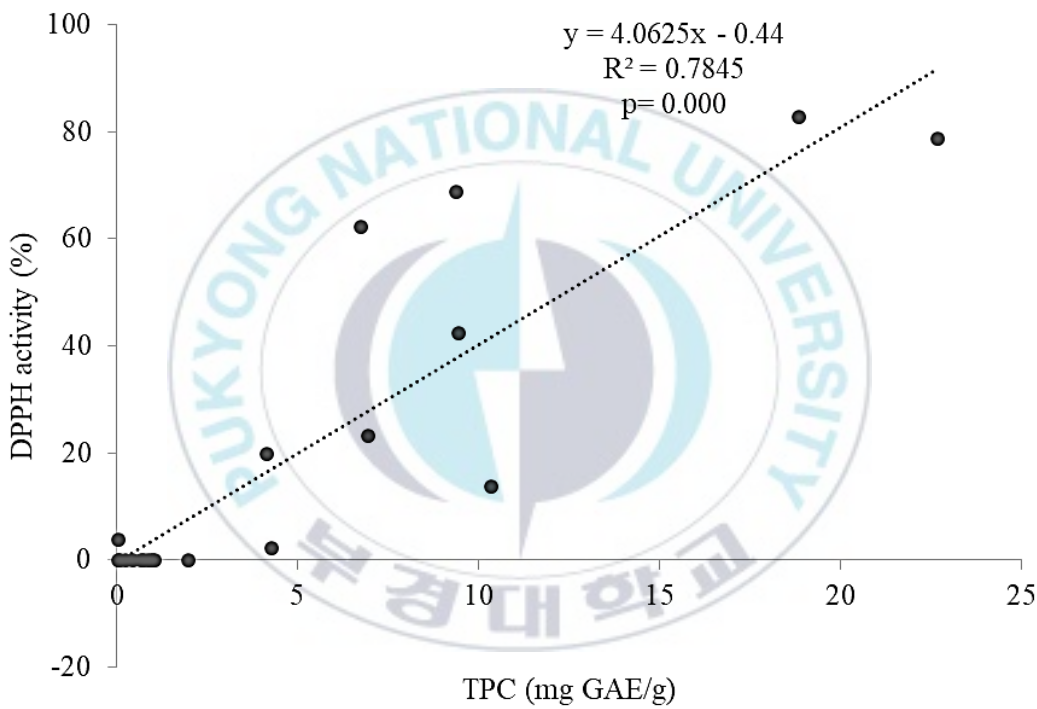

Fig. 11: Correlation of DPPH scavenging activities with TPC

3.5. *In vitro* antidiabetic activity

As α-amylase is a key enzyme involved in the starch breakdown in the human body, the inhibition of this enzyme significantly decreases the postprandial increase of blood glucose level after a carbohydrate diet by slowing down the passage of carbohydrates into the bloodstream; therefore, it could be an important strategy for managing type 2 diabetes (Kwon et al., 2008). However, most synthesized α–amylase inhibitors showed excessive inhibition and caused adverse side effects in gastrointestinal function, leading to increased demand for food-based natural α-amylase inhibitors (Kwon et al., 2008), Many kinds of plant- and animal-based polyphenols, flavonoids (Umeno, Horie, Murotomi, Nakajima, & Yoshida, 2016), and polysaccharides (Getachew, Cho, & Chun, 2018) have both antioxidant and antidiabetic activities.

An *in vitro* α–amylase inhibitory assay was performed to assess the antidiabetic potential of the extracts, and results are presented in **Table 3.** α–Amylase inhibition percentages in SWE of viscera at 110°C and spines at 150°C were similar and showed the highest values, whereas SE of gonads showed the lowest. However, n-hexane, ethyl acetate, and methanol extracts from the different body parts of the sea urchin *E. mathaei* showed dose-dependent higher α–amylase inhibition percentages than those in the current study (Soleimani, Moein, et al., 2016). Also, the *in vivo* antidiabetic activity of diethyl ether extracts from the sea urchin (*P. lividus*) shell and spines were reported (Mohamed, Soliman, & Marie, 2016; Soliman, Mohamed, & Marie, 2016). Thus, the antidiabetic activity of sea urchin extracts in the current study and other studies could be considered for the development of alternative therapy for diabetes.

3.6. *In vitro* anti-inflammatory activity

Inflammation is a typical protective reaction of the body to an injury or infection that can be characterized by heat, redness, pain, swelling, and loss of functions (IQWIG, 2006). Generally, non-steroidal anti-inflammatory drugs are used for the management of inflammatory conditions; nevertheless, they have several adverse effects (Vonkeman & van de Laar, 2010). Hence, attention to natural products has increased (Aswad et al., 2018; Dutartre, 2016).

Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. In inflammatory diseases, autoantigens may be generated as a result of *in vivo* protein denaturation (Murugan & Parimelazhagan, 2014). Thus, agents that can inhibit protein denaturation would be suitable for anti-inflammatory drug development. Also, *in vitro* protein denaturation inhibition assays are used in anti-inflammatory drug screening because of their user-friendliness and lack of ethical issues (Williams et al., 2008). In the current study, protein denaturation inhibition activities of the extracts were analyzed *in vitro*, and results are presented in **Table 3**. Aspirin (1 mg/mL) was used as the standard anti-inflammatory drug and showed 31.49% inhibition of protein denaturation. The highest inhibition of protein denaturation was recorded from SWE viscera at 150°C (37.13 ± 9.94%), but it did not significantly differ from SWE viscera at 110°C (22.89 ± 1.73%), shells at 190°C (31.81 ± 1.06%) and 230°C (23.42 ± 8.28%), gonads at 190°C (29.23 ±

0.07%) and 230°C (29.81 ± 7.93%), and SE gonads (30.12 ± 3.88%). %). In addition, the standard had a similar inhibition range as the extracts.

According to Soleimani, Moein, et al. (2016), different organic solvent extracts from the gonads, spines, and shells of the sea urchin *E. matheai* showed dose-dependent protein (BSA) denaturation inhibition activity. In that study, 1.25 µg/mL concentration of extracts and aspirin showed similar results with the current study. Also, methanol extracts from gonads of *S. variolaris* showed *in vivo* anti-inflammatory activity higher than that of Ibuprofen (Francis & Chakraborty, 2020b). Further, *in vivo* anti-inflammatory activity was present in the extracts of sea urchin shells and spines (Brasseur et al., 2017; Francis & Chakraborty, 2020a; Hou, Carne, McConnell, Bekhit, et al., 2020; Hou, Carne, McConnell, Mros, et al., 2020; Sheean et al., 2007).

In ancient Chinese medicine, sea urchins were used for treating inflammatory diseases (Shang et al., 2014). The findings of the current study along with recent studies support this medical application by demonstrating anti-inflammatory activity in sea urchin extracts.

3.7. *In vitro* anticancer activity

Within the past few decades, several new experimental anticancer agents derived from marine sources were identified and moved into preclinical and clinical trials (Pereira et al., 2019). A few studies have demonstrated the anticancer properties of several sea urchin extracts against different cancer cell lines (Brasseur et al., 2017; Liu et al., 2007; Sheean et al., 2007). The current study assessed the anticancer properties of SWE of *S. variolaris*

against HeLa cell lines. As previous studies suggested antioxidant capacity as a clue for anticancer potential (Willcox et al., 2004), the extracts that demonstrated the highest antioxidant activity in each body part (SWE of viscera at 150°C, spines at 150°C, shells at 190°C, gonads at 190°C and SE of viscera) were purposively selected for anticancer screening. Intial srceenind results are presented in **fig. 12**. Among them SE of viscera did not show considerable anticancer activity (IC_{50} >> 2000 µg/mL) when compared to SWE. Therefore, only SWE were used for further analysis . Among them, SWE of spines at 150°C claimed the highest HeLa cell inhibition by showing the lowest IC_{50} value (767.47 µg/mL) (Fig. 2b). SWE of shells at 190°C demonstrated IC_{50} =1604.41 µg/mL (Fig. 2c), whereas the IC_{50} values of gonads at 190°C (Fig. 2d), and viscera at 150°C (Fig. 2a), were higher than 1,900 µg/mL. The SWE of spines at 150°C was also tested against HUVEC and HEK-293 cell lines but did not show significant cytotoxic activity (**Table 4**).

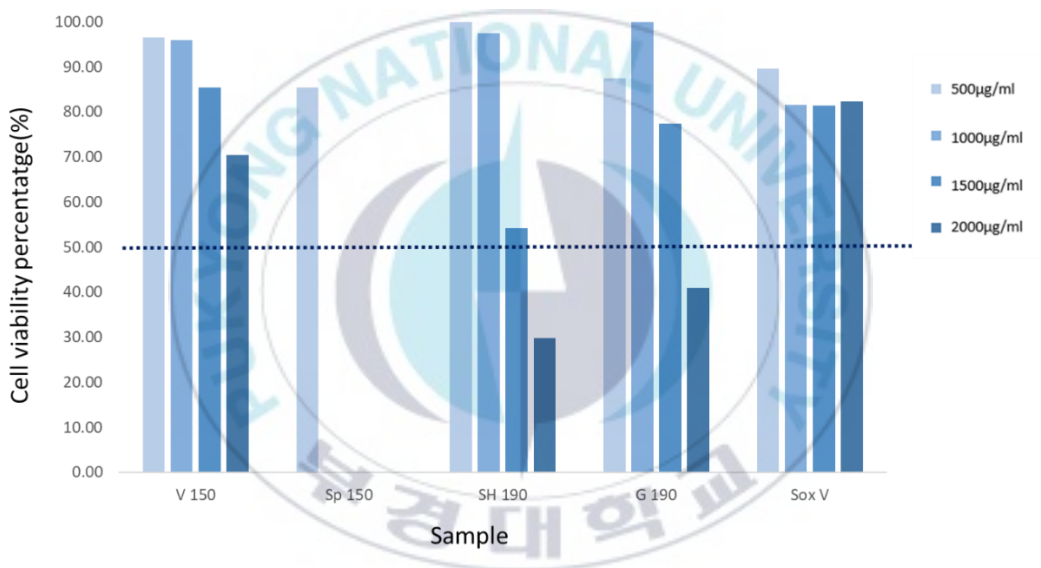

Fig. 12: Results of the initial screening of the anticancer activity of the extracts against Hela cells.

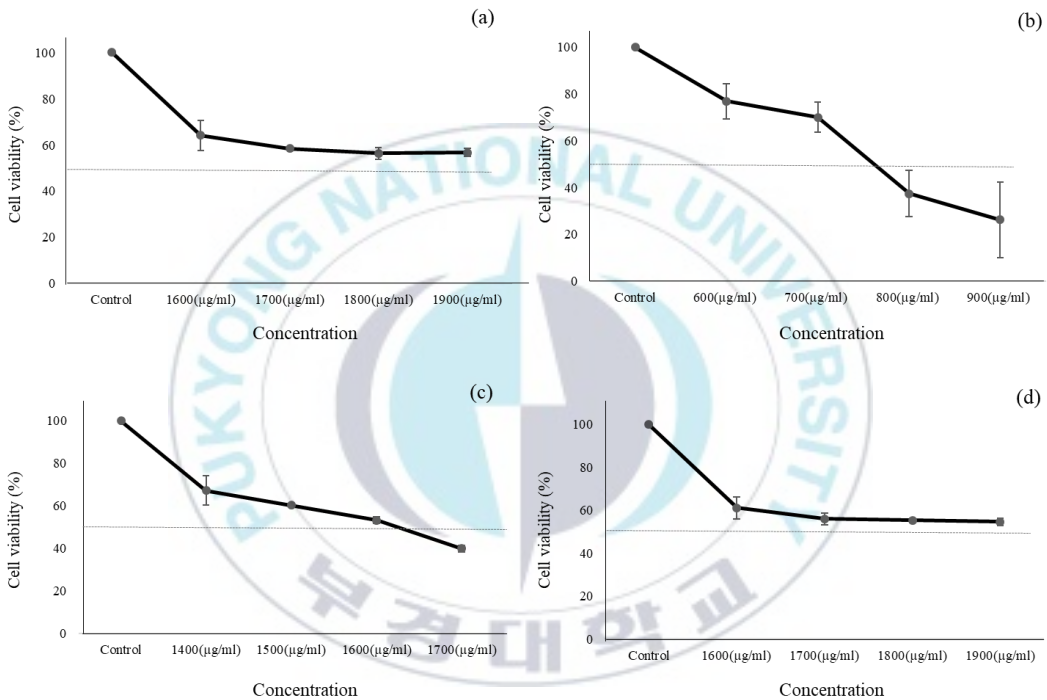

Fig. 13. HeLa cells viability (%) to different SWE of *Stomopneustes variolaris.* (a) Viscera 150°C, (b) Spines 150°C, (c) Shells 190°C (d) Gonada 190°C.

Table 4. HUVEC and HEK-293 Cell viability (%) of SWE of spines at 150°C.

Cell line	Treatment	Cell viability (%)
HUVEC	control	100
	400 μg/mL	94.26
	600 μg/mL	99.89
	800 μg/mL	95.72
	1000 μg/mL	94.10
HEK-293	Control	100
	400 μg/mL	92.37
	600 μg/mL	92.58
	800 μg/mL	89.68
	1000 μg/mL	83.07

Also, spinochromes fractionated from organic solvent extracts of sea urchin spines and shells showed 50% HeLa cell inhibition at lower concentrations (<500 µg/mL) than those in the current study (Brasseur et al., 2017).

3.8. HPLC analysis of phenolic compositions

The SWEs that demonstrated the highest TPC in each body part (viscera at 190°C, spines at 150°C, shells at 190°C, and gonads at 230°C) were selected for HPLC analysis and compared with the SEs of each body part. The identified phenolic compounds and their concentrations in each sample were presented in **Table 5**. Gallic acid, 5-HMF, chlorogenic acid, ferulic acid, and 5-MF were the identified phenolic compounds (Fig. S5). Gallic acid was identified in both SWE and SE of every analyzed body part. Results demonstrated that SWE is more effective in the recovery of a higher diversity of phenolic compounds in higher concentrations than SE. Phenolics have been considered for their powerful antioxidants (Fernandez-Panchon, Villano, Troncoso, & Garcia-Parrilla, 2008), anti-cancer (Gomes et al., 2003), anti-diabetic (Ali Asgar, 2013), and anti-inflammatory (Hyun, Ko, Kim, Chung, & Kim, 2015) properties. Therefore, the bioactive potentials described above could be ascribed to phenolic compounds or their synergetic effect with other compounds in the extracts.

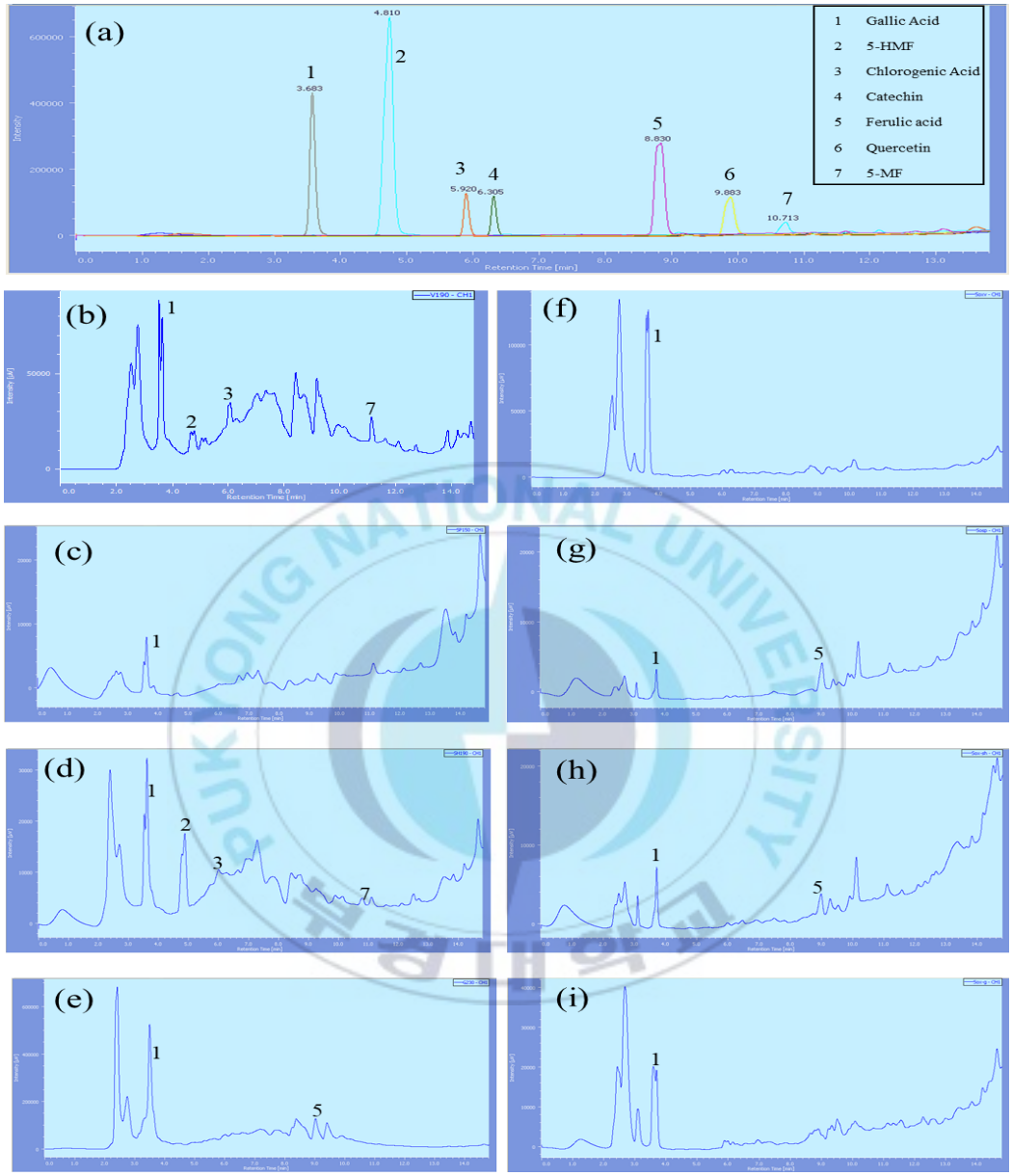

Fig. 14. HPLC profiles of the phenolic composition analysis. (a) Standards, (b) SWE of viscera at 190˚C, (c) SWE of spines at 150˚C, (d) SWE of shells at 190˚C, (e) SWE of gonads at 230˚C, (f) SE of viscera, (g) SE of spines, (h) SE of shells, (i) SE of gonads

Table 5. Concentrations of phenolic compounds

Extraction method	Sample	Phenolic compounds (mg/g of sample)				
		Gallic acid Mean ± SD	5-HMF_ Mean ± SD	Chlorogenic acid Mean ± SD	Ferulic acid Mean ± SD	5-MF Mean ± SD
SWE	Viscera 190℃	4.964 ± 0.021	0.026 ±0. 013	0.174 ±0.012	ND	0.014 ± 0.002
	Spines 150℃	0.045 ± 0.010	ND	ND	ND	ND
	Shells 190℃	1.173 ± 0.011	0.061 ± 0.011	0.039 ± 0.001	ND	0.036 ± 0.001
	Gonads 230℃	27.380 ± 0.015	ND	ND	0.266 ± 0.021	ND
SE	Viscera	0.691 ± 0.012	ND	ND	ND	ND
	Spines	0.042 ± 0.011	ND	ND	0.004 ±0.001	ND
	Shells	0.023 ±0.001	ND	ND	0.004 ± 0.001	ND
	Gonads	0.171 ± 0.011	ND	ND	ND	ND

SWE: Subcritical water extraction; SE: Soxhlet extraction; ND: Not detected; Each value is presented as mean ± SD (n = 3)

4. Conclusions

The outcomes of the current study demonstrated that SWE was able to recover more bioactive compounds from sea urchin *S. variolaris* than SE, including total phenolics, flavonoids, proteins, and sugars. In addition, the *in vitro* antioxidant, antidiabetic, anti-inflammatory, and anticancer activities of SWE were higher than those of SE. However, the SWE of viscera and gonads showed higher bioactive compound recovery compared to shells and spines. Despite the yield of the bioactive compound, all extracts demonstrated considerable bioactivities. Therefore, SWE could be useful for the extraction of bioactive compounds from sea urchin *S. variolaris* for advanced applications.

For example, it can be used in the food industry as an animal extract-based food additive or a nutrition supplement including phenolics, flavonoids, polisachcharaides and proteins. Also the antioxidant capacity of the SWE showed the potential appilaction in the cosmetic industry as an active ingredient. Antioxidant, antidiabetic, anti-inflammatory, and anticancer activities demostrated the potential use of these extracts in phamacutical industry so that develop thereputic treatements for diabetic, cancers and infalamatory diseases. Additionally, these extracts can be incoperated as an active ingredient in bioactive film formulation to produce active food packageing materials.

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Abstract (in Korean)

청정 및 기존의 기술을 이용하여 성게에서 (*Stomopneustes variolaris*) 추출한 생체활성 화합물의 *in vitro* 특성분석

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요 약

성게(로)는 영양성분이 높은 고가의 명품 식품이다. 그러나 고나드는 성게 전체 몸통의 작은 부분으로, 먹을 수 없는 껍질, 가시, 내장액, 장기 등은 정기적으로 폐기물로 버려진다. 가공 산업의 이러한 높은 폐기물 발생은 환경 문제에 기여한다.따라서 이러한 폐자재는 보다 생산적으로 사용될 필요가 있습니다. 이 연구는 110°C, 150°C, 190°C, 230°C 및 삭슬렛 추출에서 아임계수 추출임계 이하 용수 추출(SWE)을 사용하여 *Stomopneustes variolaris*의 내장, 척추, 껍질 및 고나드에서 얻은 추출물의 체외 생체 활동을 조사했다. 고나드로부터 230°C에서 페놀리(22.68±0.05mg GA/g), 플라보노이드(27.11±0.10mg RE/g), 단백질(40.25±0.84mg BSA/g)의 가장 많은 양이 기록되었다. 최대 당 함량(23.38±1.30mg 포도당/g)은 150°C에서 내장(viscera)이었다. 230°C에서 고나드는 가장 높은 DPPH 활성(78.68±0.18%)을 보였다. 150°C의 비세라(viscera)는

$ABTS^{+}$(98.92±1.27%)와 항염증 활동(37.13±9.94%)이 가장 높았다. 110°C에서 비세라가 가장 높은 아밀라아제 억제(42.46±0.83%)를 주장했다. 가시는 150°C에서 가장 높은 항암 활성(IC_{50} = 767.47 μg/mL)을 보였다. SWE는 생체 활성 화합물 복구에서 우수한 결과를 달성했으며 더 높은 수준의 생체 활동을 탐지했습니다($p<0.05$). 현재 연구의 결과는 SWE 추출이 총 페놀, 플라보노이드, 단백질 및 당을 포함하여 SE 추출보다 *S.variolaris* 로부터 더 많은 생물학적 화합물을 회수할 수 있었다는 것을 보여주고있다.또한, SWE 추출물의 시험관내 실험 항산화, 항비만, 항염증, 항암 활성은 SE보다 높았다. 하지만, 내장 및 생식선의 SWE 추출믈은 성게의 껍질과 가시에 비해 생체 활성 화합물의 회수율이 더 높았다. 많은 생체 활성 화합물이 회수되었고, 모든 추출물은 상당한 생체 활동을 보여주었다. 따라서 SWE 추출은 *S. varolaris*에서 생물 활성 화합물을 추출하는 데 유용하다. 예를 들어, SWE 추출물은 식품 산업에서 동물성 추출물을 기반으로 한 식품 첨가제 또는 페놀, 플라보노이드, 다당류 및 단백질을 포함한 영양 보충제로 사용될 수 있다. 또한 SWE 추출물의 항산화능력은 화장품 산업에서 활성 성분으로서의 잠재적 응용 가능성을 보여준다.추출물의 항산화, 항비만, 항염증 및 항암 활성은 의약품 산업에서 추출물의 잠재적 사용 가능성을 나타내고 있으며, 비만, 암 및 염증 치료제의 개발에 사용되어질 수 있다. 또한 이러한 추출물은 활성

식품 포장 재료의 생산을 위한 생체 활성성분으로 이용될 수 있다.

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Dedication

To my parent

To my teachers

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To the citizens of Sri Lanka who uphold the promis of free public education by contributing through taxes