Thesis for the Degree of Master of Engineering

An Ethanol Extract of the Rhodophyte *Gloiopeltis furcata* Improves Exercise Performance in Mice

by

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The Graduate School

Pukyong National University

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홍조류 *Gloiopeltis furcata* 에탄올 추출물의 마우스 운동능력 증진 효과

Advisor: Professor Yong-Ki Hong

by
Lisa Andriani Sutikno

A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Engineering

in Department of Biotechnology, The Graduate School,
Pukyong National University

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August 2017

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Abstract

The red seaweed *Gloiopeltis furcata* is a perennial red alga with a wide spectrum of activities in biological systems, including antitumor, antibacterial, antioxidant and cholesterol-reducing activities. In a screening test evaluating the ability of 29 common seaweed species to enhance exercise capacity, an ethanol extract of *G. furcata* (GFE) had the most potent enhancing effect on swimming endurance and forelimb grip strength in mice. The GFE extract was administered orally to mice at dose of 0.1mg/10 μL/g of body weight for 7 days. To evaluate exercise performance, forelimb grip strength and forced swimming endurance were assessed initially and after 7 days of treatment. The result indicated that GFE concentration-dependently increased forelimb grip strength, swimming endurance, and muscle density ($p<0.05$), with an optimal concentration of 10 mg/mL of 5% Tween-80. GFE was significantly reduced blood levels of lactate and urea ($p<0.05$), while the glucose, glutathione peroxidase, superoxide dismutase, and high-density lipoprotein cholesterol levels was significantly increased ($p<0.05$). Our findings suggest that GFE improve physical exercise performance and decrease the oxidative stress induced by the exhaustive exercise.

Keywords: Exercise performance, grip strength, swimming endurance, red seaweed, *Gloiopeltis furcata*

I. INTRODUCTION

The benefit of regular physical exercise includes reducing the risk of cardiovascular disease, cancer, osteoporosis, and diabetes have been acknowledged (Leeuwenburgh and Heinecke, 2001). However, strenuous physical exercise could make the body become unable to maintain its physiological level performance or designated exercise intensity which defined as physical fatigue. It has been demonstrated that exercise leads to changes in metabolism, energy provision, increase in free radical formation that causes oxidative damage to membranes (lipid peroxidation), and various disorders in relation to bioregulatory, autonomic nervous, endocrine, and immune system (Naveen and Anilakumar, 2014).

There are three main causes that contribute to physical fatigue including energy source depletion, excess metabolite accumulation, and production of free radicals (Zhao *et al.,* 2015). At the beginning of the exercise, energy resources such as glucose and glycogen are used and may soon become exhausted (Huang *et al.,* 2013), causing the production and accumulation of metabolic products including lactic acid and ammonia in the body (Huang *et al.,* 2014). Strenuous exercise

causes oxidative stress, leading to an imbalance between reactive oxygen species (ROS) production and antioxidant defense.

All fatigue effects that mentioned above could possibly affect our daily routine activity by decreasing our work efficiency, thus in order to recover from fatigue state and enhance our work efficiency, it is important to counter the fatigue effects by anti-fatigue compound.

There has been many research that investigates about herbal medicine and natural compounds as an important resource for improving athletic ability, postponing fatigue, and accelerating the elimination of fatigue-related metabolites. However, the development of a safe and effective anti-fatigue compound is considered as an important research in order to provide dietary supplements or "tonics" as an alternative (Chi *et al.,* 2015). Some of the previously researched natural compound sources in exercise supplements come from herbal resources (Yu *et al*., 2010; Yu *et al*., 2008; Yu *et al*., 2006; Wang *et al.,* 2010; Ni *et al.,* 2013; Huang *et al*., 2012) while some other comes from animal resources such as hot water extract of leather carp *Cyprinus carpio nudus* (Lee *et al*., 2015) and hot water extract of the soft-shell turtle *Trionyx sinensis* (Harwanto *et al*., 2015). Recently, one of the unicellular algae species, *Chlorella vulgaris* was also suggested to

improve physical stamina (An *et al*., 2006). However, only a few studies have examined compounds from seaweed that could improve exercise performance.

Gloiopeltis furcata (Postels et Ruprecht) J. Agardh is a perennial red alga (Rhodophyta) which belongs to the genus Gloiopeltis of Endocladiaceae family. *G. furcata* are distributed in the north coast of Pacific, including Korea and Japan and have been traditionally consumed as medicines as well as food thickeners in China and Japan (Schachat and Glicksman, 1959). The major component of this seaweed is carbohydrates, which accounted for 56.1% of total dry mass (Tao *et al.*, 2001). The extracts from *G. furcata* have been reported with variety benefit properties, such as antibacterial (Kurihara *et al*., 1999), antioxidant (Fang *et al*., 2010), anticancer (Bae and Choi, 2007), anti-inflammatory (Niu *et al.*, 2003), and anti-proliferative activities (Park *et al.,* 2005).

Of the 29 common edible seaweed species screened for their anti-fatigue activity, the ethanol extract of *Gloiopeltis furcata* (GFE) has shown the most promising exercise performance enhancing activity. The present study has been conducted to evaluate the anti-fatigue activity of GFE using forelimb grip strength test and forced swimming

endurance test, along with the muscle density measurement and determination of blood biochemical parameters, such as serum glucose, lactate, blood urea nitrogen (BUN), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), triglyceride, high-density lipoprotein (HDL) cholesterol, and total cholesterol contents. We also evaluate the effect of various drying method as well as effect of seasonal variation on GFE to mice exercise performance. Additionally, the active compounds from GFE that suggested to have anti-fatigue activity are being identified in order to support the exploration of anti-fatigue compounds in the future.

II. MATERIALS AND METHODS

2.1. Collection and processing of seaweed samples

Twenty-nine species of marine algae samples were collected separately from Indonesia and South Korea. Seventeen seaweeds species were originated from Indonesia while twelve seaweeds species were originated from South Korea. Samples were rinsed thoroughly with fresh water to remove all the extraneous matter such as epiphytes, sand, salts, and shells. Samples were dried under shade for 5 days and then grinded into a fine powder using grinder. The powder was stored in zipper plastic bag under dark condition at 20^0C for further use. Voucher specimens were deposited in the Biochemistry and Marine Biotechnology Laboratory of Dr. Y. K. Hong at Pukyong National University, Busan, Korea.

Gloiopeltis furcata as the selected seaweed species was collected from Cheongsapo Beach, Busan, South Korea ($35^0$09'39"N, $129^0$11'38"E). To determine seasonal variations, samples were collected in spring (March-May 2016), summer (June-August 2016), autumn (September-November 2016), and winter (December 2016-February 2017). The previously mentioned process was applied to this

seaweed sample. Each month's seaweed powder was stored in different zipper plastic bag for further use.

In this experiment, five different drying methods were applied to *Gloiopeltis furcata* sample that were collected in May 2016, they were freeze drying, oven drying, sun drying, shade drying, and salting process followed by shade drying method. Freeze drying method were performed by froze the seaweed at -20ºC for 1 day and freeze dried the frozen seaweed using freeze dryer at a vacuum pressure and the temperature of -80ºC for 2 days. Oven drying method were performed by kept the seaweed sample inside a 70ºC oven for 1 day. Sun drying method were performed by drying the samples under sunlight for 3 days while shade drying method were performed by drying the samples away from sunlight for 5 days. The last method was a combination between salting process as the most common preservation method by adding salt (30% of total sample gross weight) and shade drying method.

2.2. Extract preparations and reagents

For ethanol extract, each seaweed sample was extracted by pouring 95% ethanol into an Erlenmeyer flask containing seaweed

powder with 50:1 (v/w) ratio. The mixture was kept on a shaker at 200 rpm at room temperature (RT) for 24 hours under dark condition. After shaking, the mixture was filtered with filtration set with vacuum pump through Advantec No. 2 filter paper. The filtrate was dried using evaporator (Eyela, Tokyo Rikakikai Co., LTD., Tokyo, Japan) and completely dried under stream of nitrogen gas while the residue powder was used to make water extract in the next process. For water extract, each seaweed residue powder was mixed with distilled water at the ratio of 50:1 (v/w) and boiled for 10 minutes. The mixture was then filtered using sterile cotton and centrifuged at 3000 rpm. The supernatant was then dried in 65ºC oven. The dried ethanol and water extracts were weighed, and extracts yield (w/w %) were calculated for every seaweed samples (given in Table 1). Dried extracts were stored in foil-wrapped vial at -20ºC. In this study, ethanol extract solutions of each seaweed used in treatment groups were prepared by dissolving the dried ethanol extract in 5% Tween-80. While for water extract solutions, they were prepared by dissolving the dried water extract with distilled water.

In this experiment, in order to determine glucose (AM201-K), urea (AM165-K), glutamic oxaloacetic transaminase (GOT; AM101-K),

glutamic pyruvic transaminase (GPT; AM101-K) triglyceride (AM157S-K), high-density lipoprotein (HDL) cholesterol (AM203-K), and total cholesterol (AM202-K) the assay kits were purchased from Asan Pharmaceutical (Seoul, Korea). While for lactate (K627-100), glutathione peroxidase (GPx; K762-100), and superoxide dismutase (SOD; K335-100) kits were purchased from BioVision (Milpitas, CA, USA). The other reagents used were of analytical grade and purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.3. Grouping and treatment of the mice

Male Institute of Cancer Research (ICR) strain mice, aged 6-8 weeks old, weighing 27 – 30 gram were purchased from Hyochang Science (Daegu, Korea). All animals were provided with a standard laboratory diet (Formula™ M07; Feedlab, Guri, Korea) and water *ad libitum*, and housed at room temperature (24 ± 1°C) under a 12-h light/ 12-h dark cycle at 65% humidity, with a maximum of 7 mice per cage. Mice were treated in compliance with current laws and guiding principles for the care and use of laboratory animals approved by the Animal Ethics Committee of Pukyong National University (Busan, Korea).

In the screening process, twenty-eight mice were randomly assigned to 4 treatment groups per seaweed samples (n=7, per group test) that include control group (administered with saline), positive control group (administered with octacosanol), ethanol extract group, and water extracts group. The same number of mice grouping were applied for all mice group in further experiment. Each group of mice were orally administered once per day for 7 days at a dose of 10μL/g body weight. One hour after each administration, each mouse underwent body weight measurement, forelimb grip strength test, and forced swimming test sequentially.

2.4. Forelimb grip strength test

Forelimb grip strength in mice were measured using a low-force testing system (Model-RX-10, Aikoh Engineering Co., Osaka, Japan). The force transducer is equipped with a 4x5 cm^2 metal net was used to measure the amount of tensile force from each mouse (Li *et al*., 2001). Each mouse was grasped at the base of their tail and lowered it vertically toward the metal net and slightly pulled backwards while the two forelimb grasped the bar, which triggered a counter pull. The grasping force was recorded by the grip strength meter in Newton (N).

Before extract administration, all mice were trained to perform this test for 3 days. Grip strength was measured before treatment (day 0) and 1 hour after treatment on the seventh day (day 7) with each measurement was repeated twice. Each mouse was subjected to 3 times grip test and the maximum force exerted by the mouse counter pull was used as forelimb grip strength. Each gathered data were accounted for their relative increase (RI) percentage to investigate its performance significance against data from day 0. The formula for counting relative increase is shown in eq.1.

$$RI\ (\%) = \frac{(day\ 7\ data - day\ 0\ data)}{day\ 0\ data}\ x\ 100\% \qquad \text{(eq.1)}$$

2.5. Forced swimming endurance test

Swimming endurance was measured 2 times: before treatment (day 0) and 1 hour after treatment on the seventh day (day 7) with each measurement was repeated twice. As describe in An *et al.,* (2006), each mouse were placed individually in a 1-L beaker (25cm height, 10cm diameter) with 20cm water depth maintained at 25 ± 1^{0}C. During the 6 min of the test, after a delay of 2 min, the total duration of immobility was measured during a period of 4 min. When the mouse ceased

struggling and remained floating motionless in the water, making only movements necessary to keep its head above water, it was considered to be immobile. The duration of swimming time was calculated by subtracting the total immobility time. Each acquired data were accounted for their relative increase (RI) using eq.1.

2.6. Biochemical assay

Blood samples from each mouse were collected thirty minutes after the last swimming test. Each mouse was anesthetized to death with high concentration ether in glass bottle and blood was withdrawn from the heart into syringes. Then, the serum was prepared by centrifugation at 3.000g for 15 minutes. Contents of glucose, lactate, urea, GPx, SOD, GOT, GPT, triglyceride, HDL cholesterol, and total cholesterol were measured using micro plate reader (Infinite M200 Nano Quant, Tecan, Männedorf, Switzerland).

2.7. Muscle density measurement

After blood collection and skin removal process, muscle mass measurement was performed by disarticulate the forelimb and hindlimb from scapula to carpus and from ilium to medial malleolus,

respectively. Each limb was weighed and the total volume were measured in a 10 mL syringe. The bone was weighed after boiling the limb for 1 minute and the muscle was removed. Muscle mass were calculated by subtracting total limb mass with bone mass while muscle volume was calculated by subtracting the total limb volume with bone volume. Muscle density formula is shown in eq.2.

$$Muscle\ density = \frac{muscle\ weight\ (g)}{muscle\ volume\ (mL)} \qquad \text{(eq.2)}$$

2.8. Thin-layer chromatography (TLC) and preparation of hexane-ether fraction

Analytical TLC was carried out on TLC Aluminium sheets pre-coated with silica gel 60 RP-18 F_{254} on plates (20x20cm, Merck, Darmstadt, Germany). In order to improve the separation, a small drop of 10mg/mL GFE extract was spotted onto the RP-TLC plates and developed with 10 different solvent systems as mobile phase in saturated glass chamber for 15 minutes at RT. After that, the TLC plates were dried and visualized the separated bands using a UV-light with 254nm and 365nm wavelength, and identified the existence of all organic compound using iodine staining.

Based on RP-TLC result, hexane:ether (95:5) solvent mixture was suggested as the appropriate mobile phase for GFE separation. Briefly, 4.686g of GFE extract was extracted with hexane:ether (95:5) three times and shaken by hand for 15 minutes long. The soluble part (upper part) was collected and evaporated using nitrogen dryer (G 4510E, Domnick Hunter Ltd., Gateshead, England) to yield 1.665g of GFE hexane:ether fraction (GFEHF) as depicted in Fig. 1.

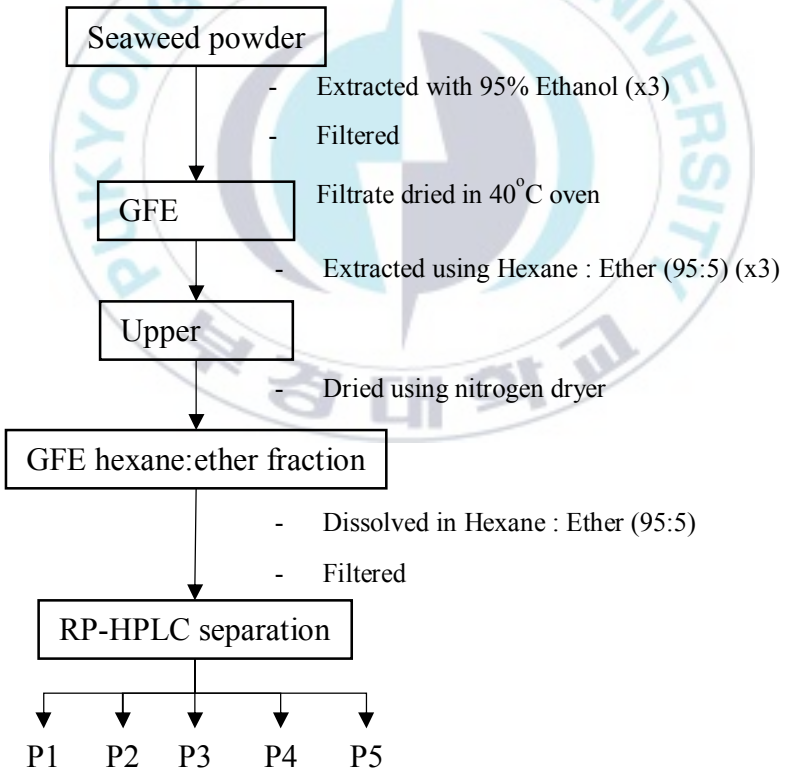


Figure 1. Schematic representation of the purification procedure to identify active compound from *Gloiopeltis furcata*

2.9. Reverse phase high performance liquid chromatography (RP-HPLC) separation

In order to separate the active compounds, RP-HPLC analysis was performed to GFEHF. The RP-HPLC system was carried out using a reverse-phase column (5μm Altima C_{18}, 250 x 10 mm, Alltech, Illinois, USA) and a guard column (5μm Altima C_{18} , 18 x 4.6 mm, Alltech, Illinois, USA), connecting with chromatography pump (Gilson piston pump 307-10SC, Gilson, Inc., USA) . The mobile phase was hexane:ether (95:5) in isocratic condition with a flow rate of 1.0 mL/min for 35 minutes per injection (15μL of 25mg/mL GFEHF). The RP-HPLC chromatogram was determined using a RI detector (Shodex RI-101, Showa Denko K.K, Tokyo, Japan). All 5 peaks from this HPLC analysis were collected for Gas Chromatography Mass Spectrometry (GC-MS) analysis and used in further mice experiment.

2.10. Statistical analysis

Data are expressed as the mean ± standard error (SE). In screening process, differences between groups were analyzed using Independent Sample t-test. $p < 0.05$ was considered to indicate a

statistically significant difference and $p < 0.001$ was considered highly significant. While for other experiment, the data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test with significant difference at $p < 0.05$. All statistical procedures were performed using IBM SPSS Statistic version 23.0 (IBM, Corp., New York, USA).

III. RESULT AND DISCUSSION

3.1. Screening of the seaweed for exercise performance enhancing activity

Many natural sources have been studied as supplements to improve exercise performance and relief fatigue symptoms. In this study, we carried out *in vivo* screening of seaweeds for their anti-fatigue activity by performing the forelimb grip strength test and swimming endurance test. For this purpose, we prepared ethanol extract and water extract from 29 common edible seaweed species and orally administered the extract to mice for 7 days.

Ethanol extract of *Hizikia fusiforme, Hormophysa triquetra, Chaetomorpha crassa, Eucheuma cottonii*, and *Gloiopeltis furcata* (Table 1), and water extract of *Eclonia cava, Caulerpa racemosa, Halimeda renschii, Acanthophora muscoides, Gigartina affinis, Glacilaria salicornia* (Table 2) had been considered to have exercise performance enhancing activities. Among them, ethanol extract of *Gloiopeltis furcata* (GFE) showed the highest relative increment on the forelimb grip strength ($p<0.001$) and swimming endurance ($p<0.01$), but not give effect to the body weight of mice. Given this findings, the

effect of GFE on exercise performance and anti-fatigue activity was further investigated.

Table 1. Screening of ethanol extract of seaweed for exercise performance enhancing activity

Seaweed Name	Country	Relative Increase (%)		
		Forelimb Grip Strength	Forced Swimming	Body Weight
Control		1.10±0.18	21.43±2.29	2.12±0.15
Octacosanol		13.24±0.91***	97.37±6.28***	9.53±0.85***
Brown Seaweed				
Costaria costata	Korea	9.81±1.11***	50.45±8.62	2.02±0.79
Dictyopteris delicatula	Indonesia	11.21±1.54***	98.16±12.36	1.33±0.24*
Ecklonia cava	Korea	7.37±0.62***	52.56±5.90	0.92±0.48*
Hizikia fusiforme	Korea	8.26±1.02***	83.76±12.68*	3.59±1.18
Hormophysa triquetra	Indonesia	6.99±0.39***	70.01±18.38*	0.70±0.14***
Padina australis	Indonesia	15.00±1.31***	77.81±27.24	2.50±0.90
Saccharina japonica	Korea	5.09±0.72	23.52±6.68	7.70±1.30**
Sargassum polycystum	Indonesia	2.51±1.79	15.28±6.87	-0.47±1.12*
Turbinaria conoides	Indonesia	4.54±0.61	88.97±25.44	0.09±0.73*
Undaria pinnatifida	Korea	1.92±0.51	33.44±9.27	3.97±0.67***
Green Seaweed				
Bornetella nitida	Indonesia	5.84±0.68***	99.10±9.89	-0.86±0.32***
Caulerpa racemosa	Indonesia	10.42±1.40***	66.29±16.04	3.54±0.76
Chaetomorpha crassa	Indonesia	9.34±1.40***	62.83±13.65*	0.86±0.64
Codium decorticatum	Indonesia	8.88±1.51***	26.90±4.55	0.58±0.33**
Codium fragile	Korea	4.20±1.70	70.87±18.54	-9.51±1.01***
Enteromorpha compressa	Korea	7.00±0.32*	38.43±5.40	-3.92±0.91***
Halimeda renschii	Indonesia	7.89±1.11***	88.33±7.34	5.74±0.75**
Monostroma nitidium	Korea	11.14±1.13***	58.43±14.39	1.26±0.64
Ulva pertusa	Korea	11.90±0.96***	38.76±5.37	0.90±0.62
Valoniopsis pachynema	Indonesia	4.70±0.90**	69.94±4.36	3.50±0.45*
Red Seaweed				
Acanthophora muscoides	Indonesia	10.92±1.40***	57.54±5.30	5.13±0.32***
Galaxaura subfruticulosa	Indonesia	6.51±0.68***	42.93±19.86	3.59±0.52*
Gigartina affinis	Indonesia	4.41±0.82**	95.53±12.90	-0.52±0.34***
Gloiopeltis furcata	Korea	10.92±1.23***	98.13±6.82**	2.77±0.27
Gracillaria sp.	Korea	6.34±0.94	54.27±10.03	0.10±1.24
Gracilaria salicornia	Indonesia	9.83±1.46***	42.59±12.63	2.86±0.37
Kappaphycus alvarezii	Indonesia	5.06±0.73***	70.78±17.66**	-0.56±0.51***
Palmaria palmata	Indonesia	3.99±0.77	18.20±1.16	-5.59±0.95***
Porphyra yezoensis	Korea	10.67±1.12***	43.65±6.01	3.09±0.63

Statistical significance compared with the control: *$p<0.05$, **$p<0.01$, and ***$p<0.001$

Table 2. Screening of water extract of seaweed for exercise performance enhancing

Seaweed Name	Country	Relative Increase (%)		
		Forelimb Grip Strength	Forced Swimming	Body Weight
Control		1.10±0.18	21.43±2.29	2.12±0.15
Octacosanol		13.24±0.91***	97.37±6.28***	9.53±0.85***
Brown Seaweed				
Costaria costata	Korea	9.24±1.48***	19.43±1.98	-1.14±0.67***
Dictyopteris delicatula	Indonesia	12.94±1.52***	11.48±1.63	1.16±0.45
Ecklonia cava	Korea	6.08±0.75***	72.02±2.57*	-2.45±0.41***
Hizikia fusiforme	Korea	4.71±0.87**	31.87±11.33	-1.67±0.49***
Hormophysa triquetra	Indonesia	7.59±1.14***	45.15±8.10	0.90±0.21***

activity

Padina australis	Indonesia	10.80±1.72***	78.62±4.43	1.18±1.04
Saccharina japonica	Korea	10.14±1.36***	56.88±5.00	6.87±1.69*
Sargassum polycystum	Indonesia	9.20±1.69**	16.75±1.52	-6.14±2.36**
Turbinaria conoides	Indonesia	1.15±0.53	23.39±2.96	-1.34±0.66***
Undaria pinnatifida	Korea	0.84±0.05	13.55±1.56	5.12±0.52
Green Seaweed				
Bornetella nitida	Indonesia	3.30±0.95**	18.43±2.79	-1.37±0.14***
Caulerpa racemosa	Indonesia	13.43±0.83***	82.53±31.68*	6.94±0.29***
Chaetomorpha crassa	Indonesia	14.74±1.47***	46.67±15.11	0.41±0.72*
Codium decorticatum	Indonesia	8.08±1.19***	58.74±7.61	0.66±0.41**
Codium fragile	Korea	3.69±1.82	60.59±8.16	-1.32±1.36*
Enteromorpha compressa	Korea	4.73±1.67	71.27±6.71**	0.05±0.03*
Halimeda renschii	Indonesia	9.53±1.38***	90.44±3.17*	5.22±0.58***
Monostroma nitidium	Korea	12.09±1.53***	48.54±4.55	4.92±0.86**
Ulva pertusa	Korea	7.55±1.35**	90.58±7.67	-1.02±0.50***
Valoniopsis pachynema	Indonesia	6.43±1.03***	49.24±4.40	5.68±0.53***
Red Seaweed				
Acanthophora muscoides	Indonesia	10.05±1.42***	75.76±2.61*	5.01±0.38***
Galaxaura subfruticulosa	Indonesia	4.00±0.95*	30.94±3.78	4.37±0.22***
Gigartina affinis	Indonesia	3.67±0.73**	78.55±2.66*	-3.00±0.52***
Gloiopeltis furcata	Korea	8.05±1.40***	62.39±6.22	6.67±0.47***
Gracillaria sp.	Korea	0.45±1.64	70.83±4.39	0.64±0.76
Gracilaria salicornia	Indonesia	9.19±1.02***	96.97±16.55*	4.34±0.14***
Kappaphycus alvarezii	Indonesia	2.99±0.85	42.63±19.03	-0.64±0.52***
Palmaria palmata	Indonesia	3.66±1.92	44.21±9.95	-6.38±0.98***
Porphyra yezoensis	Korea	3.22±0.93*	52.34±27.96	-4.67±0.69***

Statistical significance compared with the control: *$p<0.05$, **$p<0.01$, and ***$p<0.001$

3.2. Optimal concentration of *Gloiopeltis furcata* ethanol extract (GFE) for exercise enhancing activity

The effect of GFE on exercise performance and anti-fatigue activity were further investigated by orally administered GFE from 1 to 20 mg/mL concentration range to mice followed by sequence of physical test includes forelimb grip strength test, forced swimming test,

body weight and muscle density measurement, and blood biochemical evaluation.

3.2.1. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on forelimb grip strength

After 7 days of GFE treatment, the effect of GFE on forelimb grip strength are shown by relative increase percentage in saline, octacosanol, and GFE treated mice groups as shown in figure 2. In saline and octacosanol group, the relative increase was 1.7±0.3% and 12.1±0.3%. While in GFE treated mice group, the forelimb grip strength was significantly increased in a concentration dependent manner with the highest increment shown at 10 mg/mL ($p < 0.05$). The relative increase at 10 mg/mL concentration is 10.4±0.6%, 6-fold higher than saline.

The previous study had found that the level of physiological fatigue can be determined by observing energy metabolism of muscular activity. Forelimb grip strength test is a simple method designed to evaluate mouse muscle force *in vivo*, by taking advantage of the mice natural tendency to grasp a horizontal metal bar or grid while suspended by its tail (Huang *et al.,* 2012). From Figure 2 it can be observed that although slightly lower than octacosanol, 10mg/mL of

GFE had shown a significant increase in mice grip strength in day 7 which suggest that GFE extract has been proven to be able to enhance forelimb grip strength of mice.

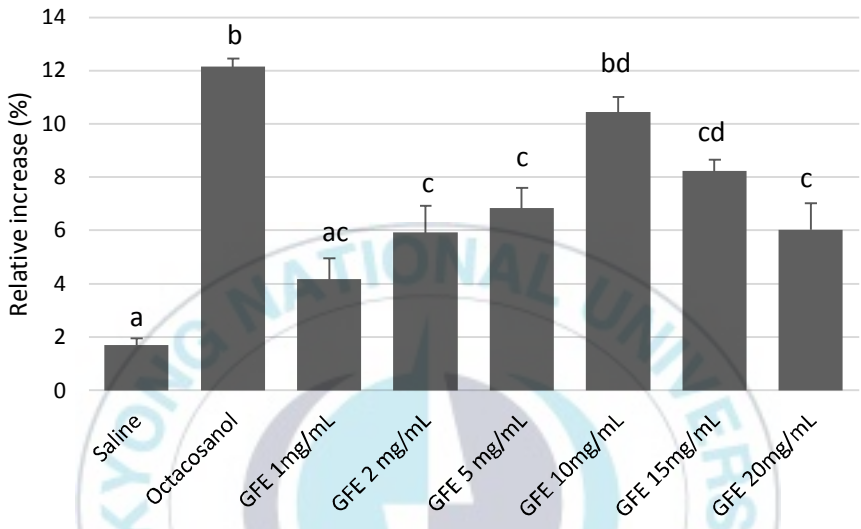


Figure 2. Effect of *Gloiopeltis furcata* ethanol extract on forelimb grip strength after 7 days of treatment. Different letters indicate significant differences among the mean after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

3.2.2. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on swimming endurance

The relative increase of swimming endurance in control and octacosanol groups on day 7 was 11.6±1.6%, and 93.5±7.2% (Figure 3). For octacosanol group, the relative increase of swimming endurance was significantly higher by 8-fold ($p < 0.05$). While for GFE treated mice group, the swimming endurance was significantly increased in a concentration dependent manner with the highest increment shown at 10 mg/mL ($p < 0.05$). The relative increase at 10 mg/mL concentration is 90.97±3.35%, 7-fold higher than saline.

Exercise endurance is an important variable in evaluating anti-fatigue treatment. In this experiment, we used swimming endurance test because it has many advantages over other types of exercise such as treadmill running. One of the advantages is that we can observe the natural behaviour of the rodents. Also, practice before the experiment is not necessarily required because rodents have a natural swimming ability, and it is assumed that they are highly motivated to avoid drowning when fatigue is imminent, assuring a high level of performance (Akira and Hiroyuki, 2005). In figure 3, it is shown that GFE extract give significant increase in mice swimming endurance

which suggest that GFE extract has been proven to be able to enhance swimming endurance capacity in mice.

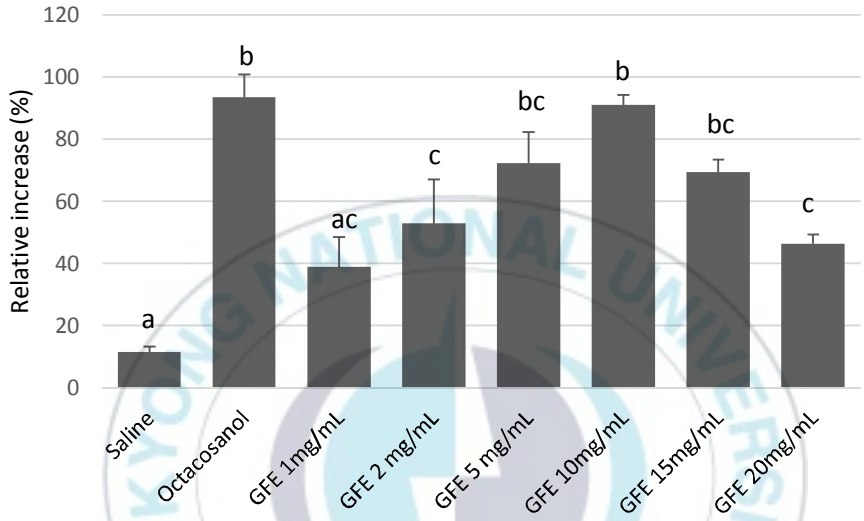


Figure 3. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on swimming endurance after 7 days of treatment. Different letters indicate significant differences among the mean after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

3.2.3. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on body weight

Before and 7 days after administration of saline, octacosanol, and GFE, changes in mice body weights were determined. The relative increase of body weight in control and octacosanol group was 2.2±0.1% and 5.5±0.2%. For octacosanol group, the relative increase of body weight was significantly higher by 2-fold ($p < 0.05$). While in mice given GFE extract, there were no significant difference in all GFE concentration ($p < 0.05$). Thus, GFE extract supplementation give a lower effect on body weight than the octacosanol.

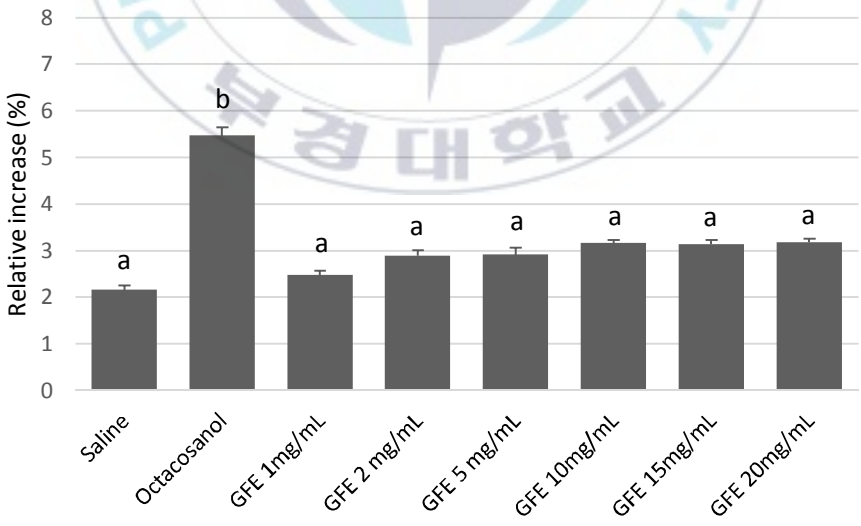


Figure 4. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on body weight after 7 days of treatment. Different letters indicate significant differences among the mean

after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

3.2.4. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on muscle density of forelimb and hindlimb

Forelimb and hindlimb muscle weight measurement of mice groups are shown in figure 5A and 5B. For both figure, octacosanol given group show significantly higher increment than saline group ($p < 0.05$). While among GFE treated mice groups, the 10 mg/mL and 15 mg/mL GFE groups shows the most significant increment compared with saline group ($p < 0.05$).

Forelimb and hindlimb muscle volume measurement of mice groups are shown in figure 6A and 6B. Forelimb muscle volume measurement for octacosanol group shows significant increment compared with saline group ($p < 0.05$). However all GFE treated groups did not show any significant difference compared with saline group. Hindlimb muscle volume measurement for all treatment groups did not show significant difference compared with saline group.

Figure 7A shows the effect of GFE supplementation on muscle density of forelimb. The muscle density in control and octacosanol group was 1.038±0.028 g/mL and 1.203±0.040 g/mL. For octacosanol group, the increment of muscle density was significantly higher than saline group ($p < 0.05$). While in mice given 10 mg/mL and 15 mg/mL

of GFE extract show significant difference compared with saline group ($p < 0.05$) with value of 1.080±0.031 g/mL and 1.078±0.031 g/mL.

GFE supplementation effect on hindlimb muscle density was shown in Figure 7B. The muscle density in control and octacosanol group was 1.058±0.020 g/mL and 1.287±0.065 g/mL. For octacosanol group, the increment of muscle density was significantly higher than saline group ($p < 0.05$). While in mice given 10 mg/mL, 15 mg/mL, and 20 mg/mL of GFE extract show significant difference compared with saline group ($p < 0.05$) with value of 1.222±0.023 g/mL, 1.227±0.042 g/mL, and 1.109±0.009 g/mL respectively. Based on these result, GFE extract has been proven to show significant increase in forelimb and hindlimb muscle density at 10 mg/mL and 15 mg/mL concentration.

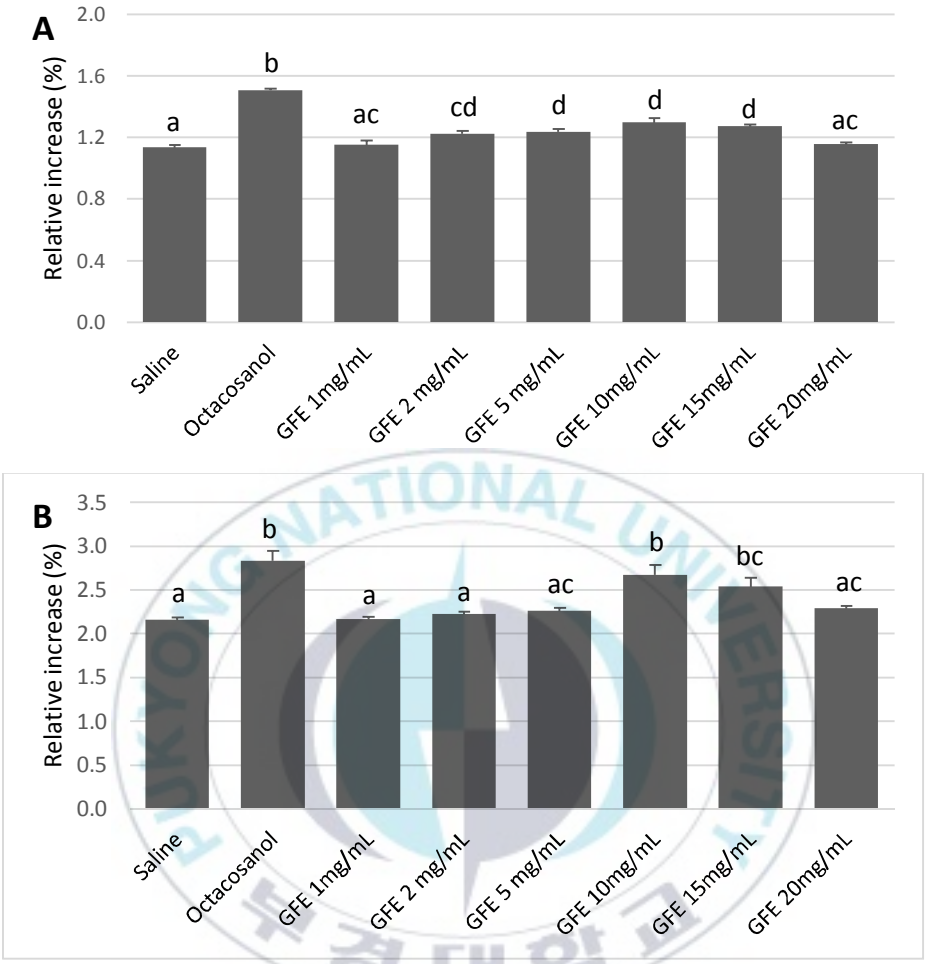


Figure 5. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on muscle weight of forelimb and hindlimb. Forelimb muscle weight (A) and hindlimb muscle weight (B) were measured 1 hour after forced swimming test at day 7. Different letters indicate significant differences among the mean after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

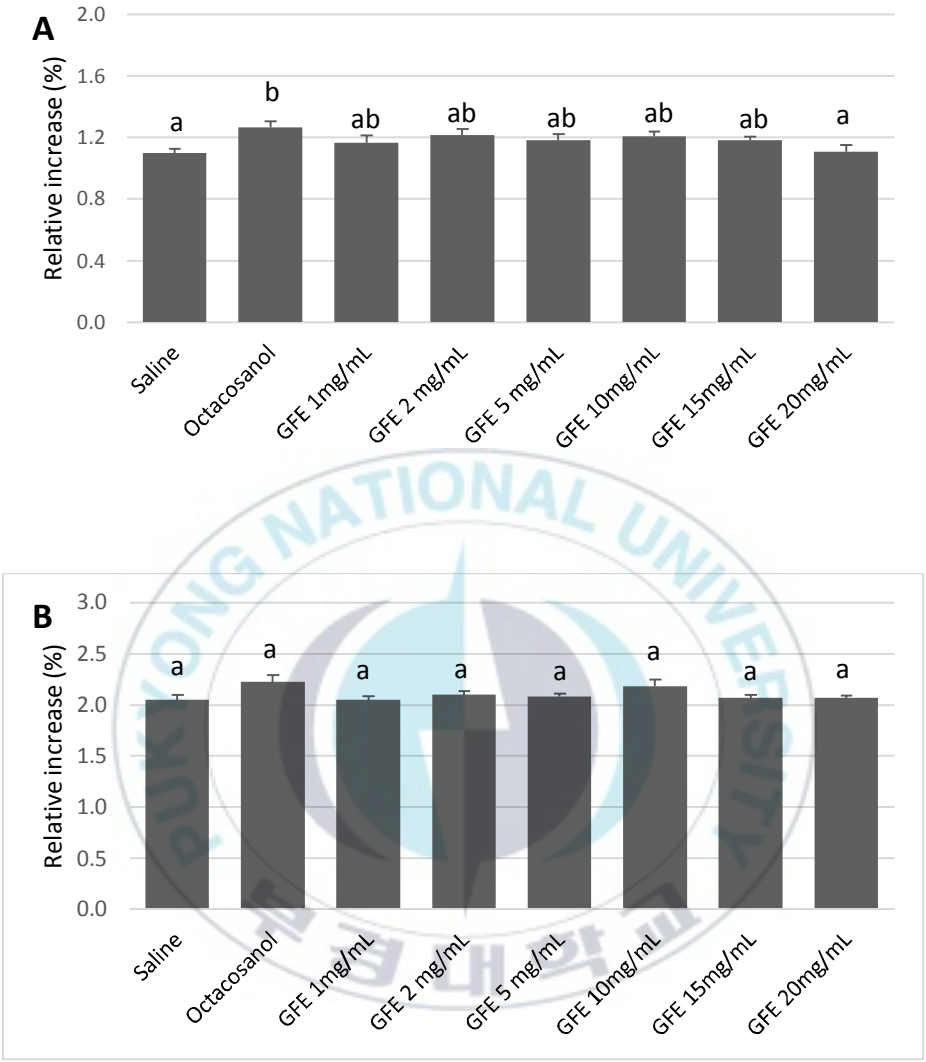


Figure 6. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on muscle volume of forelimb and hindlimb. Forelimb muscle volume (A) and hindlimb muscle volume (B) were measured 1 hour after forced swimming test at day 7. Different letters indicate significant differences among the mean after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

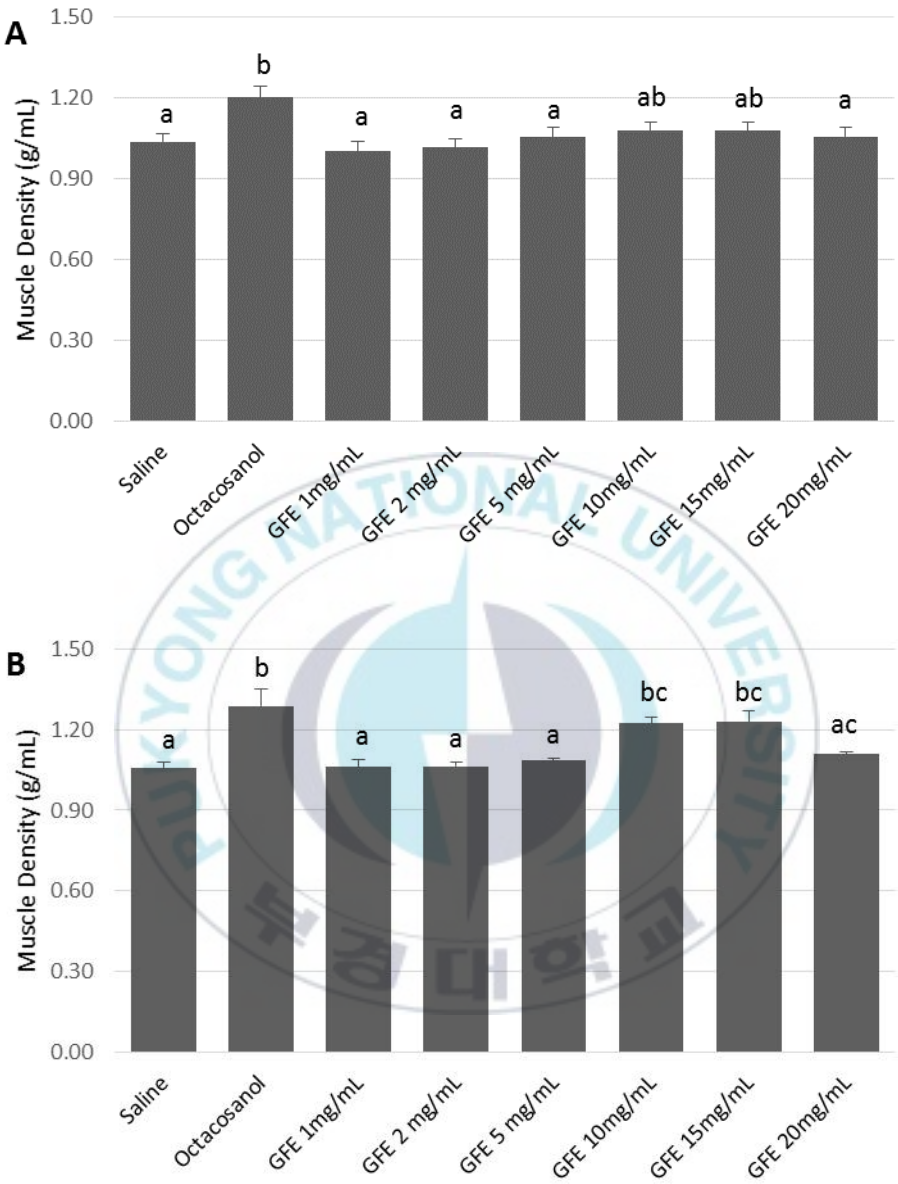


Figure 7. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on muscle density of forelimb and hindlimb. Forelimb muscle density (A) and hindlimb muscle density (B) were measured 1 hour after forced swimming test at day 7. Different letters indicate significant differences among the mean after Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean (n=12).

3.2.5. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on blood biochemical parameters

Post-exercise induced muscle fatigue can be evaluated by important biochemical indicators. After 7 days of experiment, the glucose level of octacosanol group show a significant increase with value of 14.4±0.7 mmol/L ($p < 0.05$) as shown in Table 3. While among the GFE treated group, the highest significant increment can be observed in GFE 10 mg/mL with value of 13.1±0.5 mmol/L ($p < 0.05$). For lactate level, the octacosanol and GFE group with 5 mg/mL, 10mg/mL, 15 mg/mL, and 20 mg/mL concentration shows significant decrement ($p < 0.05$). Moreover, the urea level show significant decrease both in octacosanol group ($p < 0.05$) and GFE group ($p < 0.05$).

These three biochemical variables, i.e. glucose, lactate, and urea are considered as important variable to indicate physical muscle fatigue after exercise. Energy storage and supply is also an important factor related to exercise performance. During exercise, rapid ATP consumption and energy deficiency is a critical cause of physical fatigue. Since skeletal muscle mainly catabolizes fat and carbohydrates as source of energy during exercise and glycogen act as the

predominant source of glycolysis for ATP production, glycogen storage directly affects exercise ability (Huang *et al*., 2012). Glucose levels were increased in respond to GFE treatment, thus it has been considered to enhance the release of glucose from tissue glycogen for energy recovery and maintained the homeostasis of serum glucose level.

In this experiment, the decreasing lactate level was also found in GFE treated group of mice. Usually, lactate level tends to increase during heavy exercise due to anaerobic metabolism becomes the dominant energy-producing mechanism. This condition could be overcome by the natural pathway of gluconeogenesis where lactate was converted to glucose in the liver and the excess glucose will be stored as liver glycogen. The decreasing manner of lactate level and increasing manner of glucose level indicate the occurrence of liver gluconeogenesis during heavy exercise. Moreover, the decrement of lactate level was associated with their contribution as energy substrates (Ren *et al*., 2011).

Table 3. Effect of *Gloiopeltis furcata* ethanol extract (GFE) on blood biochemical parameters after the forced swimming test

Extract	Glucose (mmol/L)	Lactate (μmol/mL)	BUN (mmol/L)	GPx (mU/mL)	SOD (mU/mL)	GOT (IU/L)	GPT (IU/L)	Triglyceride (mmol/L)	HDL Cholesterol (mmol/L)	Total Cholesterol (mmol/L)
Saline	11.3±0.6^{a}	1.3±0.0^{a}	7.8±0.1^{a}	264.1±4.5^{a}	9373.3±121.8^{a}	21.7±0.1^{a}	11.7±0.2^{a}	1.8±0.1^{a}	1.5±0.0^{a}	4.3±0.1^{a}
Octacosanol	14.4±0.7^{b}	1.1±0.0^{b}	6.9±0.2^{b}	275.3±3.3^{a}	9395.4.1±116.9^{a}	21.7±0.3^{a}	11.6±0.3^{a}	1.7±0.0^{a}	1.8±0.1^{bc}	4.3±0.1^{a}
GFE 1mg/mL	11.3±0.3^{a}	1.1±0.0^{a}	7.6±0.1^{ac}	257.0±7.5^{a}	9468.2±116.7^{ab}	21.4±0.2^{a}	11.2±0.3^{a}	1.7±0.0^{a}	1.7±0.0^{a}	4.3±0.0^{a}
GFE 2mg/mL	11.5±0.2^{a}	1.1±0.0^{a}	7.4±0.1^{ac}	269.9±3.8^{a}	9479.9±38.4^{ab}	21.6±0.3^{a}	11.8±0.4^{a}	1.8±0.0^{a}	1.7±0.1^{ab}	4.3±0.1^{a}
GFE 5mg/mL	12.8±0.5^{ab}	1.0±0.0^{b}	7.4±0.2^{ac}	281.3±4.8^{a}	9481.7±78.1^{ab}	21.3±0.3^{a}	11.2±0.1^{a}	1.8±0.0^{a}	1.7±0.0^{ab}	4.3±0.0^{a}
GFE 10mg/mL	13.1±0.5^{ab}	1.0±0.0^{b}	7.0±0.0^{bc}	321.5±14.8^{b}	10017.6±84.8^{b}	21.5±0.6^{a}	11.1±0.4^{a}	1.8±0.0^{a}	1.8±0.0^{c}	4.3±0.0^{a}
GFE 15mg/mL	13.0±0.2^{ab}	1.0±0.2^{b}	7.1±0.2^{bc}	384.8±11.0^{b}	10016.5±262.5^{b}	21.4±0.7^{a}	11.0±0.4^{a}	1.8±0.0^{a}	1.8±0.0^{bc}	4.2±0.1^{a}
GFE 20mg/mL	13.0±0.1^{ab}	1.0±0.0^{b}	7.3±0.2^{ac}	351.9±4.6^{b}	10029.9±118.6^{b}	21.5±0.4^{a}	11.3±0.3^{a}	1.7±0.0^{a}	1.8±0.0^{bc}	4.3±0.1^{a}

GPx, glutathione peroxidase; SOD, superoxide dismutase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HDL, high-density lipoprotein. Values are the mean±SE (n=12). Different letters indicate significant differences among the mean after Tukey's test (p<0.05).

The blood urea nitrogen (BUN) test is a primary test to evaluate the renal function. Urea is produced in the liver as the end product of protein and amino acid metabolism (An *et al*., 2006). One of the result of amino acid metabolism is ammonia and the increment in ammonia level is related to both peripheral and central fatigue during exercise (Huang *et al*., 2012). The result of urea evaluation in this experiment shows significant decrement in octacosanol and GFE treated group, thus it can be considered that GFE extract positively affect the performance endurance.

Heavy exercise can lead to the production and accumulation of excess reactive oxygen species (ROS), which increase the level of oxidative stress in the body and contribute strongly to fatigue. Oxidative stress is defined as the imbalance between oxidant and antioxidant levels in the body due to either the increased production of reactive oxygen species or the decreased level of the antioxidant defense. Because the antioxidant defense becomes weaker during fatigue, the improvement of antioxidant enzyme activities can help to fight against fatigue (Ren *et al.,* 2011). The activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as the major antioxidant enzymes, were used as a measure of the anti-fatigue effect

of GFE extract. GPx and SOD levels in GFE group with concentration of 10 mg/mL, 15 mg/mL, and 20 mg/mL were significantly higher than both octacosanol and control group ($p < 0.05$). As explained thoroughly in previous study (Lee *et al.*, 2015), leather carp supplementation also enhanced the GPx and SOD level thus improve exercise performance in mice.

GOT and GPT activities which indicate liver toxicity, were all within the normal range (0 – 40 IU/L). While serum HDL cholesterol levels in GFE 10 mg/mL was 1.8±0.0 mmol/L ($p < 0.05$) which was significantly higher than the saline-treated and octacosanol-treated group. After 7 days of treatment the triglyceride and total cholesterol levels stayed relatively constant since the mice did not accumulate triglyceride or total cholesterol, which indicate the sign of obesity at high concentrations.

From all above evaluation results, GFE 10 mg/mL concentration is suggested as the best concentration to enhance exercise performance, therefore we use this concentration in further experiments.

3.3. Effect of various drying method on exercise performance

Various drying method has been routinely used to preserve the nutritional values and prolong shelflife in food products including grains, fruit, vegetables, marine products, and meat products (Ratti, 2001). Raw food products have a wide range of moisture contents, from as low as 18–25% in grains to as high as 90% or more in some fruits, which need to be reduced to a level at which microbial spoilage and deterioration resulting from chemical reactions is significantly inhibited thus enable them for long term storage (Jangam, 2011).

Before being used in any nutritional evaluation or industrial processing, fresh seaweeds that collected from the sea are usually dried for achieving a high quality product. Since crude extracts of the wet seaweed do not gel thus proper drying method is essential to improve seaweed nutritional values preservability for a number of years without appreciable loss of their gel content (FAO, 1976).

There are several common drying method employed in seaweed studies including sun-drying (Carrillo *et al*., 1992), oven-drying (Hamdy and Dawes, 1988), and freeze-drying (Mabeau *et al*., 1992). However, each of these methods are usually used independently on different species of seaweed. In this experiment, the best method

among five different drying methods (freeze-drying, sun, oven, shade, salting followed by shade drying) are investigated in GFE for its effect on exercise performance.

The first evaluation method of various drying effect on exercise performance is conducted by measuring the forelimb grip strength on mice. After 7 days of treatment with GFE extract that were dried using various methods, forelimb grip strength from all treatment groups were significantly increased ($p < 0.05$) as can be observed in figure 6A. The highest activity is shown in freeze-dry group with the value of 9.147±0.429% ($p < 0.05$).

The second evaluation method is conducted by measuring swimming endurance on mice. With the same manner as the previous evaluation, the result from all treatment groups shows significant increment ($p < 0.05$) of swimming endurance as can be seen in figure 6B. The highest relative increase is achieved by freeze-dry group with the value of 83.614±5.016% ($p < 0.05$).

Summarizing from above results, various drying method effect of GFE on exercise performance are ranked as follow; oven dry < 30% salt dry < sun dry < shade dry < freeze dry.

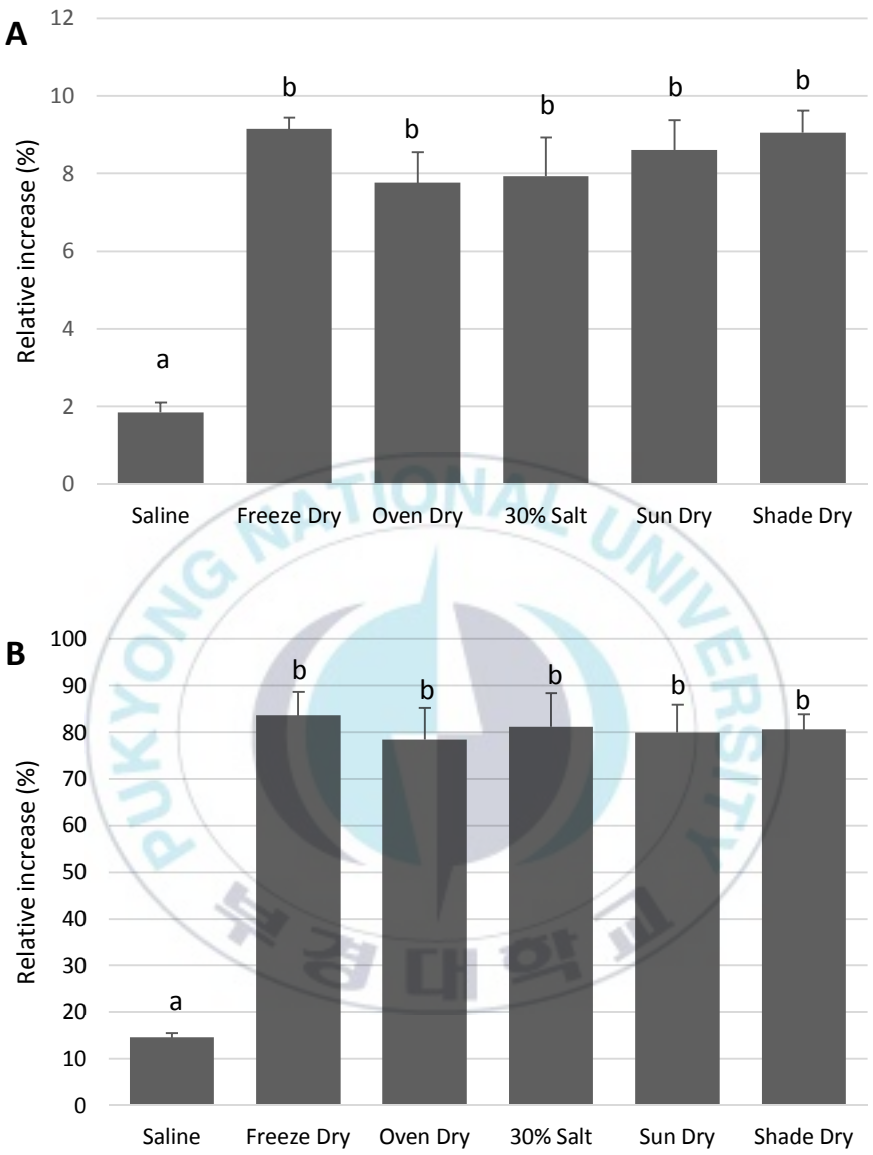


Figure 8. Effect of various drying method of *Gloiopeltis furcata* sample on exercise performance enhancing activity. Relative increase of (A) Forelimb grip strength and (B) swimming endurance, after 7 days of treatment. Different letters indicate significant differences among the mean after Tukey's test ($p<0.05$). Error bars represent the standard error of the mean (n=12).

From previous research, different drying methods have been found to have significant effects on the amount of nutritional composition extracted from seaweed. In recent years, freeze drying, have gained popularity as alternative drying methods for a variety of food products (Wang and Sheng, 2006). Freeze drying method has provided foods with the best nutritional quantity. Previous research has proven that freeze drying process had a positive effect on the total phenolic, tannins, and flavonoid compounds than oven drying (Mphahlele *et al*., 2016). Similar positive effect of freeze drying has been researched for the brown seaweed *Sargassum hemiphyllum* (Chan *et al*., 1997). However, the equipment and operating costs for freeze drying are higher and its drying capacity is smaller than those for shade, sun and oven drying.

For economic reasons, shade drying can be one of alternative drying method, since it does not need any particular equipment and can be easily applied in ordinary place. In the previous research, this method has been proven to retain the nutritional composition such as total polyphenols and antioxidants compound (Mudau and Ngezimana, 2014). In this study, shade dried samples had been observed to have a similar exercise performance enhancing activity as the freeze dried

samples, thus this drying method is highly recommended for current and further research.

3.4. Effect of seasonal variation on exercise performance

The seasonal variation highly affects the ecological condition that can stimulate or inhibit the biosynthesis of several nutrients thus influence the environmental parameters (Lobban *et al*., 1985). These environmental parameters include water temperature, salinity, light, and nutrients are related with the seaweeds nutrition contents variation (Dawes, 1998). Knowledge of the biochemical variations that take place in seaweeds during the year provide useful data for culturing conditions and the timing of harvesting for special extracts.

After observing the seaweed occurrence for this past year, it was found that *Gloiopeltis furcata* grew the most during winter and spring season but did not grow at all during autumn season. From the past research, it was reported that in Daesong-ri, Kyungsangnam-do, South Korea, the formation periods of carpospores and tetraspores of *G. furcata* was during spring and summer season (Kang and Sohn, 1992), while from the most recent study, it has been reported that the seaweed occurrence were sighted during winter and spring season in Ulsan (Choi and Rho, 2010).

After 7 days of treatment, among four seasonal groups, the spring season (March, April, and May) exhibit the highest activity by their significant increase of relative increase from both forelimb grip strength ($p < 0.05$) and swimming endurance ($p < 0.05$) result as shown in figure 7A and B. The relative increase of forelimb grip strength for March, April, and May are 9.754±0.607%, 9.522±0.267%, and 9.538±0.907% respectively, while the relative increase of swimming endurance are 72.444±7.474%, 89.024±8.340%, and 86.812±10.803% respectively.

Based on above results, the author assume that spring is the best season to harvest *Gloiopeltis furcata* in order to obtain its maximum potential for exercise enhancer. From previous studies (Banerjee *et al.*, 2009), it has been reported that seasonal changes have a significant correlation with the biochemical composition of seaweed and have a potential role in the biosynthetic pathways of seaweed. Furthermore, Polat and Ozogul (2013) reported that protein levels of seaweed were generally higher during winter and spring and lower during summer. Hence, we assume that the cellular component level of *Gloiopeltis furcata* reach its highest level during spring season. However, in this

study thorough nutrition analysis has yet to be conducted, therefore further research is encouraged to confirm this hypothesis.

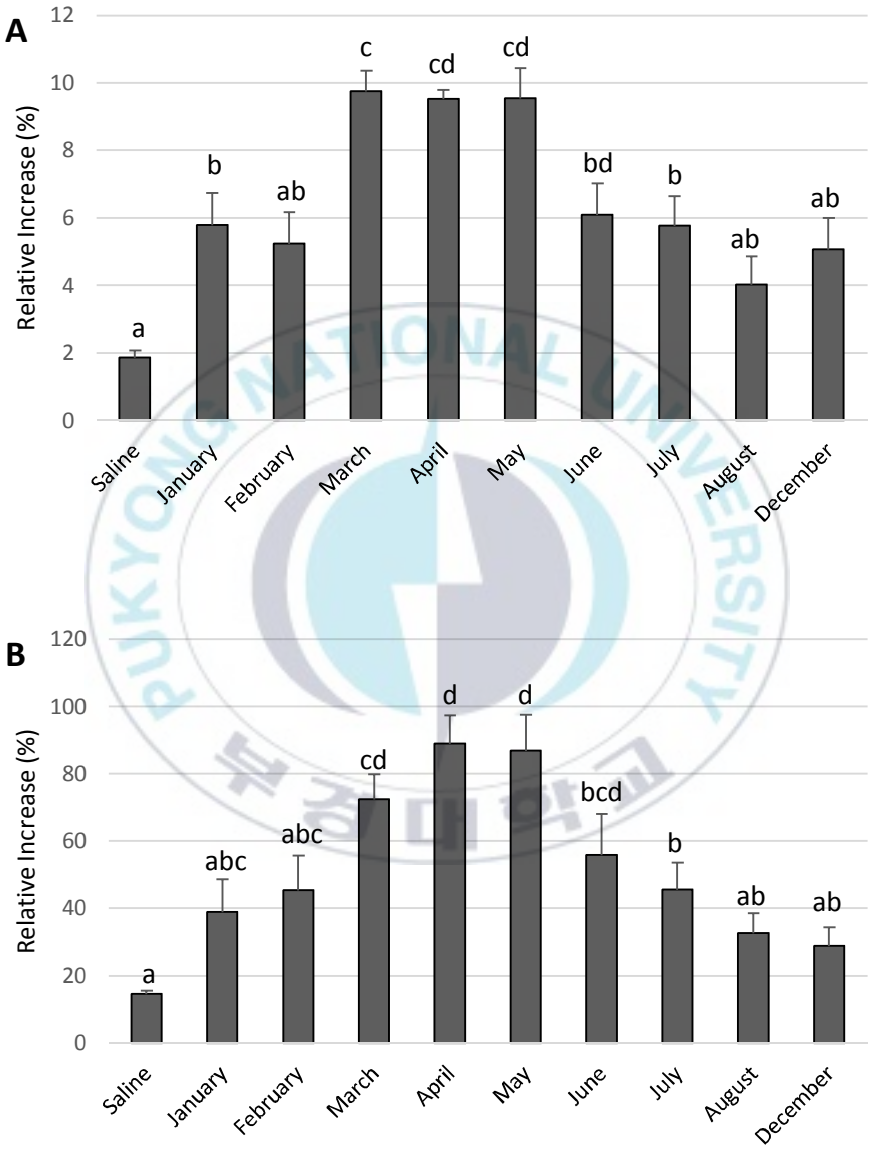


Figure 9. Effect of seasonal variation of *Gloiopeltis furcata* sample on exercise performance enhancing activity. Relative increase of (A) Forelimb grip strength and (B) swimming endurance, after 7 days of treatment. Different letters indicate

significant differences among the mean after Tukey's test ($p<0.05$). Error bars represent the standard error of the mean (n=12).

3.5. Purification process of *Gloiopeltis furcata* ethanol extract (GFE)

The active compound of GFE that responsible for exercise enhancing activity has yet been found, thus purification is a compulsory process in order to identify this compound. Therefore, I developed purification method for the identification of exercise performance enhancer agent from GFE as mentioned in Fig. 1. Based on extraction result using hexane-ether (95:5), the upper part of this sub-fraction (GFEHF) was significantly increased the forelimb grip strength ($p < 0.05$) and swimming endurance ($p < 0.05$) of mice (data not shown). Thus, the GFEHF will be used for further RP-HPLC separation.

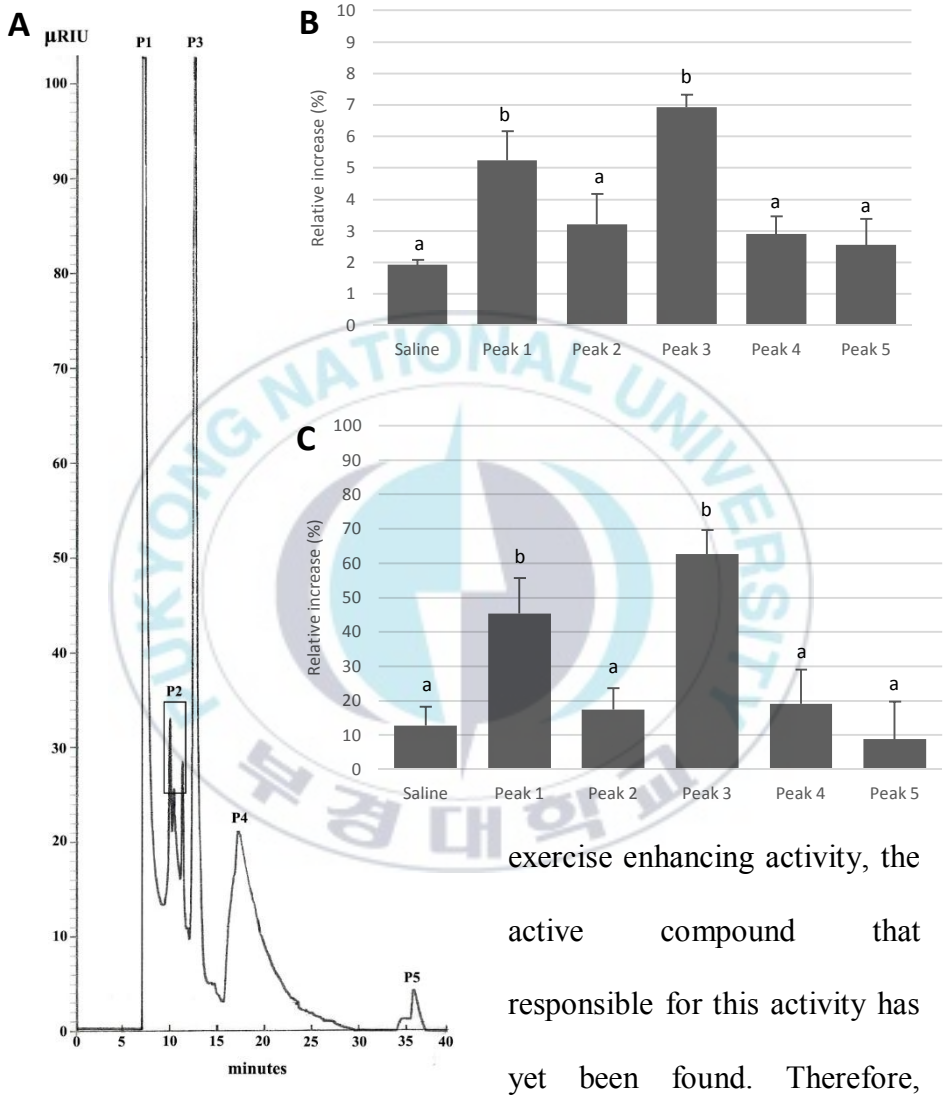
After optimization of all condition for RP-HPLC separation of the active fraction GFEHF, the injected fraction showed 5 distinct peaks in the chromatogram (Figure 8A). Therefore, each peak was collected and subjected to employ for their exercise performance enhancing activity in mice. In order to identify the active compound in the injected fraction, each peak (0.25 mg/mL) was orally administered to mice for 7 days and evaluated by forelimb grip strength and

swimming endurance measurement. Of the 5 peaks, P1 and P3 possessed the effective exercise performance enhancing effect, where they significantly increased the forelimb grip strength ($p < 0.05$) and swimming endurance ($p < 0.05$) of mice (Figure 8B and 8C). The relative increase of forelimb grip strength in P1 and P3 are 8.957±1.163% and 6.936±0.394% while the relative increase of swimming endurance are 75.507±7.498% and 62.707±6.985%.

The result above suggest that further research is needed to be done on the first and third peak of RP-HPLC result in order to confirm the active compound that responsible for exercise enhancing activity from GFE extract.

In conclusion, GFE extract is suggested to be one of the most promising supplement to enhance exercise performance based on all proven evaluation results in this study that include forelimb grip strength, swimming endurance, muscle density, and blood parameters. Additionally, we also has proven that freeze and shade drying method were the most recommended drying method for *G.furcata*. Furthermore, spring season is the most suitable time to harvest this seaweed for its maximum exercise enhancing activity.

Although the mentioned results have been very strongly proven that GFE has



exercise enhancing activity, the active compound that responsible for this activity has yet been found. Therefore, further research is still needed to ensure the specific compound from P1 and P3 of RP-HPLC result.

Figure 10 Effect of different potential compounds in *Gloiopeltis furcata* ethanol extract (GFE) on exercise performance enhancing activity. (A) Representative RP-HPLC chromatogram obtained from GFE. (B) Relative increase of forelimb grip strength, and (C) relative increase of swimming endurance after 7 days of treatment. Different letters indicate significant differences among the mean after Tukey's test ($p<0.05$). Error bars represent the standard error of the mean (n=12).

REFERENCES

Akira, W., Hiroyuki, T., and Futoshi, O. 2005. Expression of vascular endothelial growth factor and its receptors in heart tissue following short-term swimming training. *Int J Sport Health Sci* 3: 91-99.

An, H.J., Seo, S.W., Sim, K.S., Kim, J.S., Kim, E.H., Lee, M.O., Park, H.S., Han, J.G., Lee, E.H., Um, J.Y., Hong, S.H., and Kim, H.M. 2006. Antifatigue effect of *Chlorella vulgaris* in mice. *Kor J Food Nutr* 19(2): 169-175.

Bae, S.J. and Choi, Y.H. 2007. Methanol extract of the seaweed *Gloiopeltis furcata* induces G2/M arrest and inhibits cyclooxygenase-2 activity in human hepatocarcinoma HepG2 cells. *Phytother Res* 21: 52-57.

Banerjee, K., Ghosh, R., Homechaudhuri, S., Mitra, A. 2009. Seasonal variation in the biochemical composition of red seaweed (*Catenella repens*) from Gangetic Delta, northeast coast of India. *J Earth Syst Sci* 118 (5): 497-505.

Carrillo, S., Castro, M.I., Perez-gil, F., Rosales, E., and Manzano, R.E. 1992. The seaweed (*Sargassum sinicola* Setchel & Gardner) as

an alternative for animal feeding. *Cuban J Agric Sci* 26: 177-181.

Chan, J.C.C., Cheung, P.C.K., and Ang, P.O. 1997. Comparative Studies on the Effect of Three Drying Methods on the Nutritional Composition of Seaweed *Sargassum hemiphyllum* (Turn.) C. Ag. *J Agric Food Chem* 45: 3056-3059.

Chi, A., Li, H., Kang, C., Guo, H., Wang, Y., Guo, F., and Tang, L. 2015. Anti-fatigue activity of a novel polysaccharide conjugates from Ziyang green tea. *Int J Biol Macromolec* 80: 566-572.

Choi, C. G. and Rho, H. S. 2010. Marine Algal Community of Ulsan, on the Eastern Coast of Korea. *Kor J Fish Aquat Sci* 43(3): 246-253.

Dawes, C.J. 1998. *Marine Botany*. John Wiley & Sons, Inc., New York, p. 480.

FAO. 1976. Production, trade and utilization of seaweeds and seaweed products. *FAO Fisheries Technical Paper* No. 159; Food and Agriculture Organization of the United Nations: Rome, p 8.

Fang, Z., Jeong, S.Y., Jung, H.A., Choi, J.S., Min, B.S., and Woo, M.H. 2010. Anticholinesterase and antioxidant constituents from *Gloiopeltis furcata*. *Chem. Pharm. Bull* 58 (9): 1236-1239.

Hamdy, A.E.A. and Dawes, C.J. 1988. Proximate constituents and lipid chemistry in two species of Sargassum from the west coast of Florida. *Bot Mar* 31: 79-81.

Harwanto, D., Lee, G.H., Park, S.M., Choi, J.S., Kim, M.R., and Hong, Y.K. 2015. Oral administration of a hot water extract of the softshell turtle (*Trionyx sinensis*) improves exercise performance. *Prev Nutr Food Sci* 20 (2): 133-136.

Huang, C.C., Hsu, M.C., Huang, W.C., Yang, H.R., and Hou, C.C. 2012. Triterpenoid-rich extract from *Antrodia camphorata* improves physical fatigue and exercise performance in mice. *Evid Based Complement Alternat Med* 2012: 364741.

Huang, W.C., Lin, C.I., Chiu, C.C., Lin, Y.T., Huang, C.Y., Wen, K.C., Chiang, H.M., Huang, C.C., and Hsu, M.C. 2013. Hepatoprotective effects of *Ixora parviflora* extract against exhaustive exercise-induced oxidative stress in mice. *Molecules* 18: 10721–10732.

Huang, W.C., Lin, C.I., Chiu, C.C., Lin, Y.T., Huang, W.K., Huang, H.Y., and Huang, C.C. 2014. Chicken essence improves exercise performance and ameliorates physical fatigue. *Nutrients* 6: 2681–2696.

Jangam, S.V. 2011. An overview of recent developments and some R&D challenges related to drying of foods. *Drying Technol* 29: 1343–1357.

Kang, R. S. and Sohn, C. H. 1992. Formation Periods of Carpospores and Tetraspores of 16 Red Algae in Daesongri, Kyungsangnam-do, Korea. *Bull Korean Fish Soc* 25(5): 392-398.

Kurihara, H., Goto, Y., Aida, M., Hosokawa, M., and Takahashi, K. 1999. Antibacterial activity against cariogenic bacteria and inhibition of insoluble glucan production by free fatty acids obtained from dried *Gloiopeltis furcata*. *Fish Sci* 65: 129-132.

Lee, G.H., Harwanto, D., Park, S.M., Choi, J.S., Kim, M.R., and Hong, Y.K. 2015. Hot water extract of leather carp (*Cyprinus carpio* nudus) improves exercise performance in mice. *Prev Nutr Food Sci* 20(4): 246-252.

Leeuwenburgh, C. and Heinecke, J.W. 2001. Oxidative stress and antioxidants in exercise. *Curr Med Chem* 8: 829-838.

Li, X., Mohan, S., Gu, W., Wergedal, J., and Baylink, D.J. 2001. Quantitative assessment of forearm muscle size, forelimb grip strength, forearm bone material, density, and forearm bone size

in determining humerus breaking strength in 10 inbred strains of mice. *Calc Tissue Int* 68(6): 365-370.

Lobban, C.S., Harrison, P.J., Duncan, M.J. 1985. *The Physiological Ecology of Seaweeds.* Cambridge University Press, Cambridge.

Mabeau, S., Cavaloc, E., Fleurence, J., and Lahaye, M. 1992. New seaweed based ingredients for the food industry. *Int Food Ingred* 3: 38-45.

Mphahlele, R.R., Fawole, O.A., Makunga, N.P., and Opara, U.L. 2016. Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of pomegranate peel. *BMC Complement Altern Med* 16: 143.

Mudau, F.N. and Ngezimana, W. 2014. Effect of different drying methods on chemical composition and antimicrobial activity of bush tea (*Athrixia phylicoides*). *Int J Agric Biol* 16: 1011-1014.

Naveen, S. and Anilakumar, K.R. 2014. *Anti-fatigue effect of Agaricus bisporus extract in rats.* Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8): 185-191.

Ni, W., Gao, T., Wang, H., Du, Y., Li, J., Li, C., Wei, L., and Bi, H. 2013. Anti-fatigue activity of polysaccharides from the fruits of

four Tibetan plateau indigenous medicinal plants. *J Ethnopharmacol* 150: 529-535.

Niu, R., Fan, X., and Han, L. 2003. A screening for the anti-inflammatory effect of algal extracts. *Oceanol Limnol Sin* 34(2): 150–154.

Park, S.Y., Jung, B.M., Choi, Y.H., and Bae, S.J. 2005. Growth inhibition effects of cancer cell lines by *Gloiopeltis furcata* fractions in vitro. *J Korean Soc Food Sci Nutr* 34(6): 771-775.

Polat, S. and Ozogul, Y. 2013. Seasonal proximate and fatty acid variations of some seaweeds from the northeastern Mediterranean coast. Oceanologia 55 (2): 375-391.

Ratti, C. 2001. Hot air and freeze-drying of high-value foods: a review. *J Food Eng* 49:311–9.

Ren, J., Zhao, M., Wang, H., Cui, C., and You, L. 2011. Effects of supplementation with grass carp protein versus peptide on swimming endurance in mice. *Nutrition* 27: 789-796.

Schachat, R.E. and Glicksman, M. 1959. *Some lesser known seaweed extracts.* Industrial Gums, Academic Press, New York, USA.

Tao, P., Xu, Q.L., Yao, J.G., and Gao, X. 2001. An analysis of nutrient components of thirteen kinds of seaweeds for food in Dalian

coastline. *J Liaoning Normal Univ* (*Nat Sci Ed*) 24: 406-410.

Wang, J., Li, S., Fan, Y., Chen, Y., Liu, D., Cheng, H., Gao, X., and Zhou, Y. 2010. Anti-fatigue activity of the water-soluble polysaccharides isolated from *Panax ginseng* C. A. Meyer. *J Ethnopharmacol* 130: 421-423.

Wang, J. and Sheng, K. 2006. Far-infrared and microwave drying of peach. *LWT – Food Sci Technol* 39(3): 247–255.

Yu, B., Lu, Z.X., Bie, X.M., Lu, F.X., and Huang, X.Q. 2008. Scavenging and anti-fatigue activity of fermented defatted soybean peptides. *Eur Food Res Technol* 226: 415-421.

Yu, F., Lu, S., Yu, F., Feng, S., McGuire, P.M., Li, R., and Wang, R. 2006. Protective effects of polysaccharide from *Euphorbia kansui* (Euphorbiaceae) on the swimming exercise-induced oxidative stress in mice. *Can J Physiol Pharmacol* 84: 1071-1079.

Yu, F.R., Liu, Y., Cui, Y.Z., Chan, E.Q., Xie, M.R., McGuire, P.P., and Yu, F.H. 2010. Effects of a flavonoid extract from *Cynomorium songaricum* on the swimming endurance of rats. *Am J Chin Med* 38: 65-73.

Zhao, X.N., Liang, J.L., Chen, H.B., Liang, Y.E., Guo, H.Z., Su, Z.R., Li, Y.C., Zeng, H.F., and Zhang, X.J. 2015. Anti-fatigue and antioxidant activity of the polysaccharides isolated from *Millettiae speciosae* Champ. Leguminosae. *Nutrients* 7: 8657-8669.

ACKOWLEDGEMENTS

First of all, I would like to give my greatest praise to Jesus Christ for His continuous blessing and guidance for me in finishing this Master Thesis through the help of all respected and beloved people around me. Although I could not possibly give my gratitude to all people who help me in writing this Thesis, at least I want to express it to the people without whom this thesis might not have been completed.

Firstly, to my advisors in Biochemistry Laboratory, **Professor Yong Ki Hong** who already gave me so many great opportunities through my Master course in Pukyong National University. He didn't only give me lots of seaweed related knowledge, but also abundance of experience in order to encourage me improving my skills. I feel really grateful to have him as my advisor due to his intense care and nurture which made me felt welcomed in his laboratory as a part of his precious family. And also I would like to thank **Professor Sung Hyun Hong** for his endless support since the beginning and through my master course study. He gave me lots of very powerful encouragement and guidance to finish my study, especially with the scientific writing method through his class.

I wish to thank all professors on my thesis evaluation committee and also all professors in biotechnology department of PKNU for their dedication and willingness to share their expertise with me through very interesting and challenging courses during these past two years.

I also want to give my appreciation and gratitude for **NIIED** that has chosen me as the grantee of the best scholarship program in Korea thus let me finish my Master degree without any financial difficulties.

I also want to give my deepest gratitude for my beloved family, especially my mother, who always support me in every hardship that I faced along these past three years studying in Korea. And also to my father, who always remind about the essence and importance of life through his great wisdom. And to my older brother and younger sister who always give me their greatest advice and prevent me from making wrong decisions.

In this chance I also want to give millions of thanks to my lovely boyfriend, who always support me emotionally through his endless love, care, and understanding through hard times and good times during my master course study.

And finally, I also would like to thank all my friends and Biochemistry Laboratory members for their support during my study in this laboratory. Special thanks for **Ms. Gong Hyeon Lee**, as our lab captain that took care all of administrative stuff for our lab and support me as a friend. Thanks for **Mr. Sylvester** for sharing his wisdom and knowledge during my study. Thanks for **Dr. Md. Mohibullah Ph.D** who always encourage me during the hard times. And also for my Indonesian friends, **Mr. Ardi Ardiansyah** and **Mr. Gabriel Tirtawijaya** for their support, encouragement, and accompaniment as same-nation fellows.

ABSTRACT (In Korean)

홍조류 *Gloiopeltis furcata* 에탄올 추출물의 마우스 운동능력 증진 효과

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초록

Gloiopeltis furcata (불등풀가사리)는 항암, 항균, 항산화 및 콜레스테롤 감소 등의 광범위한 생물학적 활성을 지닌 다년생의 홍조식물이다. 운동능력 향상 실험을 위한 29 종류의 일반 해조류 스크리닝 실험의 결과 *G.furcata* 의 에탄올 추출물(GFE)이 수영 지구력과 앞발 악력 실험에서 가장 높은 효과를 보였다. 이 연구에서는 GFE 추출물을 7 일 동안 0.1mg/10μL/g of body weight 의 투여량으로 마우스에 경구투여하였다. 운동능력 향상을 측정하기 위해 수영지구력 및 앞발 악력 실험을 첫날 시행하였으며 이후 7 일간 GFE 의 경구투여 후 다시 한번 더 시행하였다. 결과는 GFE 의 농도에 따라 수영지구력, 앞발 악력 및 근밀도($p<0.05$)가 향상되었으며 이때 최적농도는 (GFE)10mg/(5% Tween-80)mL 이였다. 또한, 실험 대조군과의 비교를 통해 GFE 가 혈중 젖산과 요소($p<0.05$)의 농도를 줄여주는 것을 관찰했으며, 반면에 glucose, glutathione peroxidase, superoxide dismutase, high-density lipoprotein cholesterol 은 유의미하게 높아짐($p<0.05$)을 관찰하였다. 이 연구결과 GFE 는 육체적 운동능력을 향상시키며 격한 운동으로 인한 산화스트레스를 감소시킨다는 사실을 알 수 있다.