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Identification and Biological Activities of the Phenolic Compounds in *Eisenia arborea*

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Abstract

Four polyphenols were isolated and purified from a brown alga *Eisenia arborea*. These phlorotannin compounds showed strong radical scavenging and some enzyme inhibitory activities. All of the compounds showed strong antioxidative, acetylcholinesterase and butyrylcholinesterase inhibitory, and tyrosinase inhbibitory activities at 100 μg/mL. Dieckol and PFF inhibited butyrylcholinesterase, a new target for the treatment of Alzheimer's disease, very strongly even at 10 μg/mL, more strongly than AChE. These two compounds also effectively inhibited tyrosinase. These results support the potential of developing natural antioxidants and antidementia agents from the brown alga.

Keywords

Eisenia arborea, Phenolic Compounds, DPPH Radical Scavenger, Enzyme Inhibition, Isolation

1. Introduction

Eisenia arborea is a dominant species of kelp that is found on the western Pacific coast of North America, from Vancouver Island, Canada south to Mexico's Isla Magdalena and Baja California, as well as in Japan. It can be commonly found from the midtidal areas stretching to the subtidal areas. It is an edible seaweed, a source of nutrients for grazing marine invertebrates and a source of alginic acid, and a food thickener. Some of the algae have a hollow stipe above its holdfast with two branches terminating in multiple blades. *Eisenia arborea* is studied in order to predict environmental stress in oceans intertidal zones. *Eisenia arborea* with hollow stripes are believed to be evolved algae in order to increase their survival in harsh living conditions. They play a huge role in determining envi-

ronmental stress [1]. It is known that the alga has secondary metabolites such as polyphnolic compounds so called phlorotannins.

Antioxidants exert protective effects in human health against oxidative damage associated with reactive oxygen species (ROS). ROS are chemically reactive substances that can attack lipids, proteins and nucleic acids within living organisms. ROS include free radicals (O_2^- , HO·, RO· and ROO·) and neutral molecules ($^1O_2$ and H_2O_2). Even though ROS generation is a normal metabolic process [2], its excessive formation can cause peroxidation in human tissues, ultimately leading to various diseases related with aging [3]. In order to reduce the cumulative effects of ROS-related damage, the addition of antioxidants to food or cosmetic formulations has been considered. Development of novel antioxidative agents, especially from natural sources, has attracted attention in terms of their ecologically friendly properties [4].

Alzheimer's disease (AD) is associated with early substantial reductions in presynaptic markers of the cholinergic system. Specifically, the activity of choline acetyltransferase, critical in the synthesis of acetylcholine (ACh), is depleted, together with ACh levels, as cholinergic neurons are lost and cholinergic neurotransmission increasingly declines with disease progression [5] [6]. Strategies to augment cholinergic neurotransmission, which is fundamental to memory and learning processes, have thus represented the primary approach to treat AD [7]. Cholinesterase (ChE) inhibitors retard the inactivation of ACh after synaptic release and represent a mainstay treatment for AD. Whereas, ChE inhibitors provide consistent improvements in cognitive performance in mild to moderate AD [8] [9]. Preliminary study indicated that the methanol extract of *Eisenia arborea* exhibited significant DPPH radical scavenging and ChEs inhibitory activities. Therefore, we decided to carry out a phytochemical investigation of the *Eisenia arborea* extract to determine the bioactive metabolites. Described herein are the identification and biological activities of the chemical constituents in *Eisenia arborea.*

2. Materials and Methods

2.1. Plant Material

The branches of *Eisenia arborea* were collected in November 2016 from offshore of Baja California, Mexico. A voucher specimen (sample number 317) was deposited at the herbarium of Baja Kelp Talasoterapia, Ensenada. The freshly collected whole seaweed was washed with tap water immediately after collection, air-dried at room temperature in a dark room for a week. The dried alga was cut into small pieces and kept at −40˚C until use.

A Photo of *Eisenia arborea*

2.2. Extraction and Isolation

The shade dried and cut *Eisenia arborea* branches (520 g) were extracted with 100% methanol (6 L) two times at room temperature for 24 h. The gummy extract (15.5 g) was obtained after concentration of the filtered solution. The extract was fractionated into *n*-hexane (1.0 g) and 80% methanol. The 80% methanol fraction was further fractionated into chloroform (0.5 g) and 30% methanol. The 30% methanol fraction was fractionated into *n*-BuOH (2.7 g) and water (9.1 g) fraction. Since the *n*-butanol fraction showed good biological activities, it was chosen to isolate the biologically active compounds. A portion (1.5 g) of the BuOH fraction was subjected ODS column chromatography, using step-gradients starting from MeOH:H_2O = 3:7 to MeOH:H_2O = 5:5 (*n*-hexane/EtOAc to EtOAc/MeOH, 300 mL each) to provide 37 fractions (V1 - V37). These fractions were subjected to silica gel column chromatography to give four compounds. The chemical structures of the compounds were elucidated by ^{1}H NMR, ^{13}C NMR, and mass data and compared with the literature data.

2.3. Reagents and Instruments

The chemical reagents, 1,1-diphenyl-1,2-picrylhydrazyl (DPPH), electric eel acetylcholinesterase (AChE, EC 3.1.1.7), horse serum butyrylcholinesterase (BuChE, EC 3.1.1.8), acetylthiocholine (ATCh), butyrylthiocholine (BuTCh), and 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma (St. Louis, MO, USA). All solvents used were of analytical grade. UV spectra were recorded with a HP8453 UV/VIS spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100.6 MHz) nuclear magnetic resonance (NMR) spectra were recorded on a JNM-LA 400 instrument (JEOL) with chemical shift data in ppm relative to the solvent used. Merck silica gel (0.063 - 0.2 mm) was used for normal phased column chromatography. Silica gel 60 F_{254} coated on aluminum plates and ODS plate by Merck were used for thin layer chromatography (TLC) and column chromatography.

2.4. Biological Assays

Radical scavenging effect was carried out according to the method first employed by M.S. Blois [10]. Cholinesterase activity was measures by the Ellman's coupled enzyme assay [11] and tyrosinase activity was also measured by the known method [12].

3. Results and Discussion

The methanol extract was prepared from the branches of *Eisenia arborea* with a 2.9% yield. The extract was partitioned successively to provide *n*-hexane, chloroform *n*-butanol and water fractions. Each fraction was then tested for its antioxidative capacities using DPPH radical scavenging assay first reported by Blois [10]. The fractions were also tested for their AChE, BuChE, and tyrosinase inhibitory activities. **Table 1** shows the antioxidative and AChE, BuChE, and tyrosi-

nase inhibitory activities of each fraction.

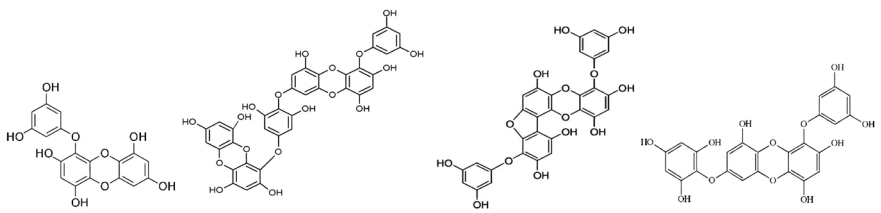
As shown in Table 1, the *n*-butanol fraction exhibited considerable antioxidative and anticholinesterases activities comparable to vitamin C and edrophonium, well known potent ChE inhibitor. The *n*-butanol fraction also showed considerable tyrosinase inhibitory activity which can be used as a skin lightening agent. Since the *n*-butanol fraction showed the highest radical scavenging and anticholinesterase activities, it was chosen for isolation of the active constituents. The *n*-butanol fraction was subjected to ODS column chromatography with gradients of *n*-hexane/EtOAc followed by EtOAc/MeOH to provide 37 fractions. The fractions showing same R_f values on TLC were pooled together and subjected to repeated column chromatography with silica gel or Sephadex LH-20, leading to the isolation of four compounds (Figure 1). The chemical structures of the isolated compounds, phlorotannins were characterized by spectroscopic data including 1D and 2D NMR spectra and MS. The phlorotannins are oligomers of phloroglucinol, the basic unit of them. All of the isolated compounds are known and previously isolated from brown seaweeds, *Ecklonia cava*, *Ecklonia stolonifera*, and *Eisenia bicyclis* [1] [12] [13] [14] [15]. Sugiura, *et al.* isolated eckol, 8,8'-bieckol, phlorofucofuroeckol A (PFF-A), and phlorofucofuroeckol B (PFF-B) to show the anti-inflammatory effects of phlorotannins from *Eisenia arborea* on mouse ear edema by inflammatory inducers [16].

The isolated compounds are oligomers of phloroglucinol, the basic structural unit of algal polyphenols so called phlorotannins. Table 2 shows that phlorotannins have strong radical scavenging, ChEs, and tyrosinase inhibitory activities. The antioxidative activity of each compound is lower than the *n*-butanol fraction implying that the active components are in that fraction. The phlorotannins have

Table 1. DPPH scavenging and enzyme inhibitory activities of the *Eisenia arborea* extract solvent fractions.

Fraction	DPPH %	AChE Inhibition %	BuChE Inhibition %	Tyrosinase Inhibition %
n-hexane	15.7	0	27.4	9.1
chloroform	21.8	12.2	30.9	14.2
n-butanol	81.3	83.1	99.5	75.3
water	18.7	0	0	8.5
control	93.0[a]	99.4[b]	85.0[b]	96.1[c]

The sample concentration was 100 μg/mL. [a]: vitamin C; [b]: edrophonium; [c]: kojic acid.



Eckol Dieckol Phlorofucofuroeckol(PFF) 7-phloroeckol

Figure 1. Chemical structures of compounds isolated from *Eisenia arborea*.

Table 2. DPPH and enzyme inhibitory activities of the isolated compounds from *Eisenia arborea*.

compound	DPPH % (1)	AChE Inhibition % (1)	BuChE Inhibition % (1)	Tyrosinase Inhibition % (1)
	DPPH % (2)	AChE Inhibition % (2)	BuChE Inhibition % (2)	Tyrosinase Inhibition % (2)
7-phloroeckol	69.7	45.1	59.7	49.4
	48.1	35.2	14.1	1.4
eckol	76.8	99.0	97.8	67.9
	58.4	40.9	48.6	13.3
dieckol	67.5	97.2	99.5	91.3
	59.5	72.3	96.2	29.3
PFF	74.0	96.1	98.0	87.3
	71.4	63.4	89.9	21.5
control	93.0[a]	99.4[b]	85.0[b]	96.1[c]

The sample concentration was (1) 100 μg/mL, (2) 10 μg/mL. [a]: vitamin C; [b]: edrophonium; [c]: kojic acid.

stronger BuChE inhibitory activity than AChE. Two phlorotannins, dieckol and PFF showed strong BuChE inhibitory activity even at 10 μg/mL. This result is consistent with our previous result [17]. According to the result IC_{50} of dieckol and PFF from *Ecklonia cava* for AChE and BuChE was 20.1, 96.3 and 2.7, 0.9 μM, respectively. The phlorotannins inhibited BuChE more strongly than AChE by 10 to 100 folds [17]. Choi and his colleagues isolated phlorotannins and showed that their tyrosinase inhibitory activities [12]. They showed PFF and dieckol inhibit mushroom tyrosinase with IC_{50} values of 33.2 and 2.16 μg/mL, respectively. Their results are consistent with our results.

Phlorotannins possess a unique structure which is not found in terrestrial plants [18]. Especially the compounds with dibenzo-1,4-dioxin skeleton which are only found in limited algae species are the most interesting ones in medicinal sense because of their relatively small size (MW 300 - 800) and their rigid structure owing to dibenzo-1,4-dioxin linkage, enabling them to strongly interact with various biological molecules [19]. Those phlorotannins share similar antioxidant properties with other well-known terrestrial polyphenols, such as catechins, quercetin, and resveratrol, due to their polyphenolic nature. However, since the characteristic dibenzo-*p*-dioxin backbone structure of the phlorotannins would provide a distinguished motif in biological interactions from those of other polyphenols, they could potentially have a variety of unique physiological features yet to be discovered.

Polyphenol compounds such as tannins and flavonoids are found in virtually all plant families. Polyphenols function as natural antioxidants via the scavenging of reactive oxygen species. The brown seaweeds have been known to have diverse secondary metabolites such as polyphenols and terpenoids. Many research groups investigated the polyphenols (phlorotannin) and their diverse bi-

ological activities isolated from *Ecklonia cava*. We were able to identify phlorotannins in another brown seaweed *Ecklonia arborea*.

In conclusion, four polyphenolic compounds were isolated and identified from the methanol extracts of *Eisenia aborea*: eckol, dieckol, phlorofucofuroeckol (PFF), and 7-phloroeckol. The isolated four compounds showed strong antioxidative, radical scavenging activity comparable to that of vitamin C The three isolated compounds except 7-phloroeckol showed very good AChE and BuChE inhibitory activities. Based on these results, *Eisenia aborea* extracts are expected to be useful antioxidative and antidementia agents with potential applications in functional food and medicinal field.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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