

The ethanol extract of the rhodophyte *Kappaphycus alvarezii* promotes neurite outgrowth in hippocampal neurons

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Abstract Neurodegenerative disorders, which affect memory, cognition, and social functions, can be treated using neurotrophic agents to support neuronal development and protect mature neurons from atrophy. We screened 34 tropical seaweed species collected from Indonesian coastal areas for their neurite-outgrowth-promoting activity (NOPA) in fetal rat hippocampal neurons in vitro. Based on the number and total length of primary neurites, red seaweeds had greater NOPA than green and brown seaweeds. The red seaweed *Kappaphycus alvarezii* showed the highest NOPA. Addition of the ethanol extract to the culture ($1 \mu\text{g mL}^{-1}$) significantly accelerated initial neuronal maturation from stage I to stage II (70 %; $P < 0.05$) within 24 h and increased the number of neurites that developed multipolar characteristics (48 %; $P < 0.05$). These results indicate that the aquaculturable carrageenan producer *K. alvarezii* might be a promising source of neurotrophic compounds to enhance memory and learning.

Keywords Hippocampal neuron · *Kappaphycus alvarezii* · Neurotrophic activity · Primary culture

Introduction

The most common age-associated dementia requires neurotrophins to prevent neuronal death or produce neuronal cells. Neurotrophins promote neurite outgrowth processes and survival. Neuronal death can be prevented by endogenous neurotrophic factors (NFs), such as nerve growth factor and brain-derived neurotrophic factors (Akagi et al. 2015). Neurotrophic factors play roles in both the development and the maintenance of the nervous system, including neuronal differentiation, maturation, and survival. Their ability to elicit pro-survival and pro-functional responses in neurons make them candidate drugs for treating neurodegenerative disorders (Weissmiller and Wu 2012).

Seaweeds are an abundant marine resource that provide important compounds to marine animals and may be used for the treatment of various human diseases. The use of seaweeds has increased due to their various advantages and ease of cultivation. In addition to their use as foodstuffs, several seaweeds have also been used as herbal medicines (Smit 2004; Kolanjinathan et al. 2014). Several studies have reported neurotrophic activities of *Sargassum fulvellum* (Hannan et al. 2012), *Gelidium amansii* (Hannan et al. 2013, 2014), *Undaria pinnatifida* (Bhuiyan et al. 2015), *Gracilariopsis chorda* (Mohibullah et al. 2015a), and *Porphyra yezoensis* (Mohibullah et al. 2015b) in primary cultures of fetal rat hippocampal neurons. Kamei and Sagara (2002) screened 300 seaweed species for their neurite-outgrowth-promoting activity (NOPA) in a rat adrenal medulla pheochromocytoma cell line (PC12D). Although most samples showed no activity, the brown alga *Sargassum macrocarpum* showed prominent NOPA. Sargaquinolic acid (Kamei and Tsang 2003) and sargachromenol (Tsang et al. 2005) isolated from *S. macrocarpum* promoted neurite outgrowth via signaling pathways in PC12D cells.

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In the present study, we screened 34 tropical seaweed species for NOPA in a primary culture of rat hippocampal neurons. The hippocampus is especially vulnerable to damage during the early stages of Alzheimer's disease (Mu and Gage 2011). In primary cultures, hippocampal neurons readily polarize, develop extensive axonal and dendritic arbors, and form numerous functional synaptic connections with one another, which are useful for determining many aspects of neuronal development (Kaech and Banker 2006; Seibenhener and Wooten 2012). Using these hippocampal neuron cells, NOPAs of tropical seaweed extracts were compared based on the number and total length of primary neurites, and the dose dependence of the effect on neuronal maturation by the seaweed with the greatest potential, *Kappaphycus alvarezii*, was investigated.

Materials and methods

Collection of seaweed samples

Thirty-four species of wild seaweed were collected from the coast of Sayang Heulang (7° 40' 08.1" S, 107° 41' 42.9" E) and Karimunjawa (5° 48' 27.6" S, 110° 22' 01.3" E), Java, Indonesia, in 2014 and 2015. Tissues were thoroughly rinsed with freshwater to remove all extraneous matter, such as epiphytes, sand, salts, and shells. The seaweed samples were identified and dried at room temperature for 3–5 days. Dried tissues were cut into small pieces, pulverized to fine powder in a grinder (HMF-340, Hanil Co., Seoul, South Korea), and stored in zipper plastic bags under dark conditions at 20 °C for further use. Voucher specimens were deposited in the author's laboratory (Y.K. Hong).

Preparation of extracts

Fine powder samples were sieved through a 36-mesh filter to obtain a small particle size. Two gram of each sample was extracted with 100 mL of 95 % ethanol in a 250-mL flask. The mixture was incubated on an orbital shaker at 200 rpm at room temperature. After 24 h, the extract was separated from the residue using a filtration set with a vacuum pump through Advantec No. 2 filter paper. The filtrate was concentrated in vacuo, and the salts contained in the extract were eliminated by repeated extractions with 100 % ethanol until no salt remained visible. After drying under a stream of nitrogen gas, the extracts were dissolved in dimethyl sulfoxide (DMSO) to 8 mg mL⁻¹ and stored at -20 °C in airtight vials until required.

Culture and treatment of primary hippocampal neurons

Primary hippocampal neurons were cultured in 24-well polystyrene plates. All cell culture reagents were from Invitrogen (USA) unless otherwise stated. All animal care and

use were in accordance with the institutional guidelines and approved by the Institutional Animal Care and Use Committee of the College of Medicine, Dongguk University, South Korea. Primary hippocampal neurons were cultured as described previously (Goslin et al. 1998; Hannan et al. 2012). Briefly, rats (Sprague–Dawley) at day 19 of pregnancy were euthanized with isoflurane, and the fetuses were collected. The hippocampi dissected from the brain were collected in Hank's balanced salt solution (HBSS) and incubated in 0.25 % trypsin in HBSS for 12 min at 37 °C to dissociate tissues. The cells were counted with a hemocytometer and plated at a density of $\sim 1\text{--}2 \times 10^4$ cells cm⁻² onto poly-DL-lysine-coated glass coverslips in 24-well plates. Cultures were maintained in serum-free neurobasal medium supplemented with B27 and incubated at 37 °C under 5 % CO₂ and 95 % air. Extract or vehicle control (DMSO, final concentration ≤ 0.5 %) was added to the culture medium prior to plating.

Image acquisition

Images (1388 × 1039 pixels) were acquired using a Leica Microscope DM IRE2 equipped with I3 S, N2.1S, and Y5 filter systems (Leica Microsystems AG, Germany) and a high-resolution CoolSNAP CCD camera (Photometrics Inc., Germany) under the control of a computer using Leica FW4000 software. The microscope was used for phase-contrast and epifluorescence microscopy. The digital images were processed using Adobe Photoshop 7.0 for Windows.

Image analysis and quantification

Morphometric analyses and quantification were performed using ImageJ version 1.48 software (<http://imagej.nih.gov/ij>) with the simple neurite tracer plug-in (National Institute of Health, Bethesda, MA, USA) and Sholl plug-in (<http://biology.ucsd.edu/labs/ghosh/software>). Morphometric parameters, such as the number of primary neurites (NPN; neurites that originated directly from the soma), total length of primary neurites (TLPN; the sum of the length of primary neurites), and length of the longest neurite (LLN), were measured. Neurons that were not intermingled with the processes of adjacent neurons were selected for analysis. Neuronal cell populations at different developmental stages and cells that exhibited unipolar, bipolar, and multipolar characteristics were also counted (Dotti et al. 1988). A vehicle control (medium with DMSO) was compared with those treated with seaweed extracts.

Statistical analysis

Data are expressed as means ± standard error. All data were checked for the normality of the distribution using a

Kolmogorov–Smirnov test. Normally distributed data were analyzed by Student’s *t* test and one-way analysis of variance followed by the least-significant-difference test with significance set at $P < 0.05$. All calculations were carried out using SPSS 17.0 software for Windows (SPSS Inc., USA).

Results

The NOPA of seaweed ethanolic extracts was evaluated based on the NPN and TLPN of embryonic hippocampal neurons. Those morphometric analyses were performed in vitro at day 2 (DIV 2). Among the 34 seaweeds tested, 14 showed neurotrophic activity based on the NPN, and 18 species showed such activity based on the TLPN (Tables 1, 2, and 3). Most of the seaweeds showed dose-dependent neurotrophic

activities. Activity increased in a dose-dependent manner at $1–10 \mu\text{g mL}^{-1}$, but it decreased or was inhibited at $20 \mu\text{g mL}^{-1}$. In our screening experiment, extracts of *Gelidiella acerosa* (EGA) and *K. alvarezii* (EKA) showed highly significant positive effects ($P < 0.001$) on NOPA in developing rat hippocampal neurons at the lowest dose, $1 \mu\text{g mL}^{-1}$ (Table 3). Upon addition of EGA, NOPAs based on NPN and TLPN were 1.31- and 1.92-fold, respectively, higher than those of the vehicle control. The addition of EKA resulted in 1.22- and 1.71-fold increases in NOPAs based on NPN and TLPN, respectively, compared with the vehicle control. Thus, we selected EKA for further evaluation because *K. alvarezii* is one of the most abundant aquaculturable carrageenophytes. Four of 10 chlorophytes (*Caulerpa racemosa*, *Codium decortatum*, *Ulva reticulata*, and *Valoniopsis pachynema*, Table 1), 1 of 8 phaeophytes

Table 1 Neurite-outgrowth-promoting activities of green seaweeds after 2 days of incubation

Species	Yield (% w/w)	Concentration ($\mu\text{g mL}^{-1}$)	NPN \pm SE (%)	TLPN \pm SE (%)
<i>Bornetella nitida</i>	0.64	1	108 \pm 5.2	113 \pm 8.6
		10	103 \pm 4.8	108 \pm 6.8
		20	95 \pm 5.2	78 \pm 6.0
<i>Caulerpa racemosa</i>	0.68	1	108 \pm 7.3	108 \pm 8.9
		10	109 \pm 5.8	121 \pm 9.1*
		20	121 \pm 6.4**	126 \pm 7.9**
<i>Chaetomorpha antennina</i>	0.52	1	98 \pm 6.3	94 \pm 8.6
		10	114 \pm 7.8	114 \pm 9.1
		20	112 \pm 5.8	105 \pm 7.5
<i>Chaetomorpha crassa</i>	1.54	1	85 \pm 4.5	100 \pm 7.1
		10	86 \pm 8.9	98 \pm 8.1
		20	Inhibitory	Inhibitory
<i>Codium decortatum</i>	1.75	1	128 \pm 5.3***	149 \pm 7.9***
		10	105 \pm 5.8	109 \pm 6.8
		20	93 \pm 6.9	61 \pm 4.2
<i>Halimeda renschii</i>	0.98	1	83 \pm 4.9	92 \pm 5.5
		10	Inhibitory	Inhibitory
		20	Inhibitory	inhibitory
<i>Ulva lactuca</i>	0.22	1	96 \pm 3.9	125 \pm 7.1**
		10	83 \pm 3.7	91 \pm 4.4
		20	79 \pm 4.5	57 \pm 3.7
<i>Ulva reticulata</i>	0.13	1	102 \pm 6.5	121 \pm 9.0*
		10	81 \pm 6.1	59 \pm 4.8
		20	88 \pm 6.8	65 \pm 6.8
<i>Ulva rigida</i>	0.20	1	107 \pm 4.3	103 \pm 6.1
		10	105 \pm 5.6	78 \pm 5.4
		20	93 \pm 6.8	54 \pm 4.8
<i>Valoniopsis pachynema</i>	1.01	1	94 \pm 3.2	110 \pm 5.2
		10	102 \pm 4.7	119 \pm 6.2*
		20	Inhibitory	Inhibitory

Activities represent the values relative to the vehicle control ($n = 60–80$ neurons for each seaweed extract)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Neurite-outgrowth-promoting activities of brown seaweeds after 2 days of incubation

Species	Yield (% w/w)	Concentration ($\mu\text{g mL}^{-1}$)	NPN \pm SE (%)	TLPN \pm SE (%)
<i>Dictyopteris delicatula</i>	0.58	1	104 \pm 5.1	95 \pm 5.6
		10	Inhibitory	Inhibitory
		20	Inhibitory	Inhibitory
<i>Dictyota dichotoma</i>	0.04	1	109 \pm 6.9	97 \pm 6.3
		10	Inhibitory	Inhibitory
		20	Inhibitory	Inhibitory
<i>Hormophysa triquetra</i>	0.55	1	92 \pm 4.3	111 \pm 5.8
		10	67 \pm 6.5	62 \pm 6.5
		20	49 \pm 5.5	45 \pm 6.2
<i>Padina australis</i>	1.29	1	125 \pm 4.5**	135 \pm 6.1**
		10	120 \pm 6.2*	139 \pm 6.7**
		20	92 \pm 4.0	74 \pm 3.4
<i>Sargassum crassifolium</i>	0.76	1	88 \pm 4.0	80 \pm 4.2
		10	63 \pm 3.1	71 \pm 4.6
		20	77 \pm 3.7	64 \pm 5.8
<i>Sargassum duplicatum</i>	0.47	1	115 \pm 4.5	105 \pm 4.7
		10	Inhibitory	Inhibitory
		20	Inhibitory	Inhibitory
<i>Sargassum polycystum</i>	0.86	1	98 \pm 3.4	74 \pm 4.7
		10	Inhibitory	Inhibitory
		20	Inhibitory	Inhibitory
<i>Turbinaria conoides</i>	0.71	1	97 \pm 4.9	103 \pm 7.1
		10	78 \pm 3.3	85 \pm 5.5
		20	71 \pm 4.2	50 \pm 3.9

Activities represent the values relative to the vehicle control ($n = 60\text{--}80$ neurons for each seaweed extract)

* $P < 0.01$; ** $P < 0.001$

(*Padina australis*, Table 2), and 11 of 16 rhodophytes (*Acanthophora muscoides*, *Acanthophora spicifera*, *Amphiroa fragilissima*, *Euclima spinosum*, *Gelidium latifolium*, *Gracilaria coronopifolia*, *Gracilaria heteroclada*, *Gracilaria salicornia*, *Gracilaria tenuistipitata*, *Gracilaria verrucosa*, and *Palmaria palmata*, Table 3) were other seaweed species that exhibited activity. Most rhodophytes (81 %) exhibited greater NOPA compared with chlorophytes (40 %) and phaeophytes (13 %). Furthermore, *Acanthophora spicifera*, *E. spinosum*, *Gracilaria tenuistipitata*, and *K. alvarezii* showed marked NPN activity, and *Acanthophora muscoides*, *Acanthophora spicifera*, and *Gelidiella acerosa* showed considerable TLPN activity. Additionally, *Halimeda renschii* and *Sargassum polycystum* exhibited marked inhibition of NPN and TLPN.

Because *K. alvarezii* had the greatest NOPA and biomass, we further investigated the extract of this seaweed. First, to evaluate the concentration dependency of the effect, 0.1 to 60 $\mu\text{g mL}^{-1}$ EKA was added to hippocampal neuron cultures. Typical micrographs of a culture grown for 2 days are shown in Fig. 1a. Growth parameters—the NPN, TLPN, and LLN—peaked at 1 $\mu\text{g mL}^{-1}$ ($P < 0.05$) and decreased gradually at

higher doses (Fig. 1b). Therefore, EKA was added to neuron cultures at 1 $\mu\text{g mL}^{-1}$ in subsequent experiments.

EKA influenced the initial development of neurons using the optimal dose, 1 $\mu\text{g mL}^{-1}$. Neurites formed during initial neuronal differentiation function as precursors for dendrites and axons in mature neurons. Neurons are categorized into the following developmental stages (Fig. 2a): lamellipodia (stage I), transition (fan-shaped process; stage 1.5), and minor processes (stage II). The number of neurons at each maturation stage differed significantly after 24-h incubation ($P < 0.05$). In cultures to which EKA was added, 70 % of the cells were in developmental stage II, and only 13 % were in stage I; however, ~46 % of the cells in the vehicle group were in stage II, and 27 % were in stage I (Fig. 2b, (1)). At 48 h, the number of neurons in stage II increased significantly ($P < 0.05$), to 85 % (vs. to only 64 % in the vehicle control), upon the addition of EKA to the medium (Fig. 2b, (2)). Thus, the addition of EKA to the fetal hippocampal neuron culture medium significantly accelerated the maturation of neuronal cells ($P < 0.05$). Furthermore, we counted the number of processes to determine the neuron type and categorized minor process (stage II) neurons into unipolar (at least one process),

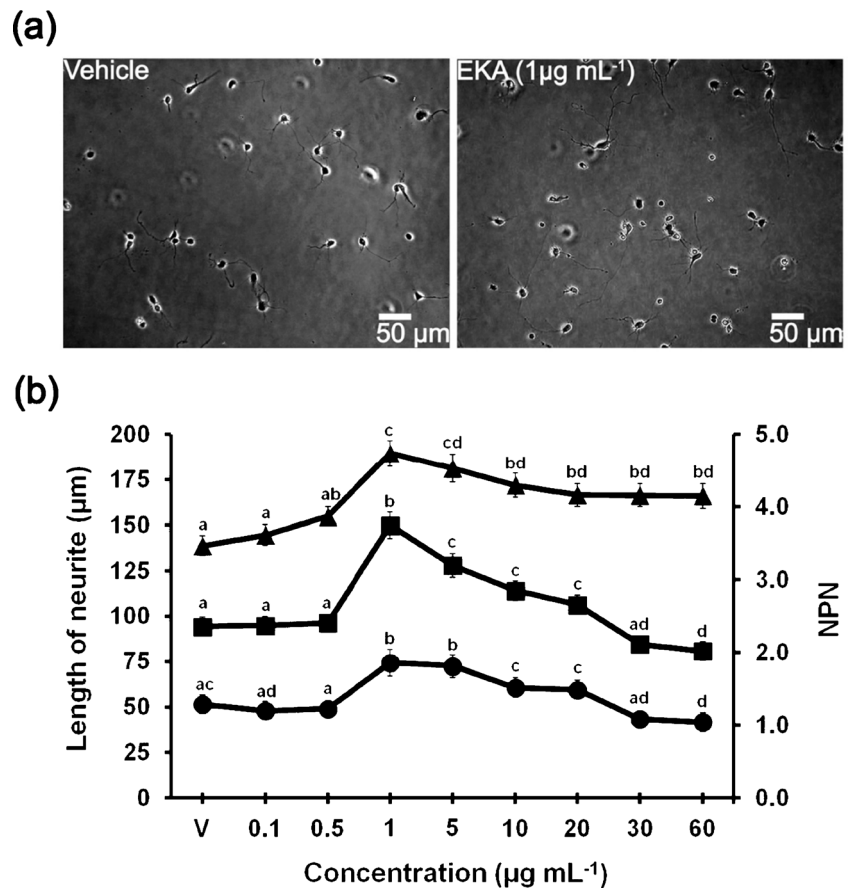
Table 3 Neurite-outgrowth-promoting activities of red seaweeds after 2 days of incubation

Species	Yield (% w/w)	Concentration ($\mu\text{g mL}^{-1}$)	NPN \pm SE (%)	TLPN \pm SE (%)
<i>Acanthophora muscoides</i>	0.66	1	114 \pm 4.8*	142 \pm 6.5***
		10	129 \pm 5.0***	197 \pm 8.7***
		20	122 \pm 4.2***	146 \pm 6.9***
<i>Acanthophora spicifera</i>	0.53	1	104 \pm 3.9	134 \pm 5.0***
		10	129 \pm 4.5***	189 \pm 8.9***
		20	136 \pm 7.4***	165 \pm 9.8***
<i>Amphiroa fragilissima</i>	0.95	1	125 \pm 6.3**	143 \pm 6.4***
		10	127 \pm 5.8***	166 \pm 9.2***
		20	128 \pm 9.0***	146 \pm 12.9***
<i>Eucheuma spinosum</i>	0.31	1	107 \pm 3.7	140 \pm 6.4***
		10	128 \pm 4.4***	154 \pm 5.9***
		20	140 \pm 5.7***	134 \pm 7.0***
<i>Galaxaura subfruticulosa</i>	0.45	1	105 \pm 5.2	88 \pm 5.5
		10	86 \pm 5.2	65 \pm 5.3
		20	115 \pm 6.1	66 \pm 6.2
<i>Gelidiella acerosa</i>	0.30	1	131 \pm 4.1***	156 \pm ***
		10	131 \pm 5.2***	103 \pm 5.0
		20	111 \pm 5.0	57 \pm 3.4
<i>Gelidium latifolium</i>	0.09	1	135 \pm 5.5***	166 \pm 9.3***
		10	123 \pm 6.0**	97 \pm 5.7
		20	Inhibitory	Inhibitory
<i>Gigartina affinis</i>	0.22	1	110 \pm 5.2	98 \pm 9.5
		10	69 \pm 6.8	52 \pm 5.4
		20	Inhibitory	Inhibitory
<i>Gracilaria arcuata</i>	0.48	1	98 \pm 3.7	103 \pm 5.6
		10	117 \pm 5.3	104 \pm 5.6
		20	119 \pm 4.5	92 \pm 4.8
<i>Gracilaria coronopifolia</i>	0.11	1	106 \pm 4.4	126 \pm 7.1**
		10	121 \pm 5.0**	128 \pm 6.7***
		20	119 \pm 7.9	82 \pm 6.5
<i>Gracilaria heteroclada</i>	0.22	1	110 \pm 5.0	140 \pm 8.9***
		10	93 \pm 5.9	73 \pm 5.1
		20	87 \pm 7.0	63 \pm 5.5
<i>Gracilaria salicornia</i>	0.52	1	98 \pm 3.9	131 \pm 7.5**
		10	109 \pm 6.3	123 \pm 7.8*
		20	100 \pm 5.1	83 \pm 3.9
<i>Gracilaria tenuistipitata</i>	0.21	1	122 \pm 6.4*	146 \pm 10.2***
		10	127 \pm 9.1**	153 \pm 14.5***
		20	135 \pm 8.8***	139 \pm 13.1**
<i>Gracilaria verrucosa</i>	0.36	1	108 \pm 5.3	111 \pm 10.0
		10	133 \pm 7.6***	155 \pm 11.6***
		20	117 \pm 10.3	115 \pm 9.6
<i>Kappaphycus alvarezii</i>	0.50	1	122 \pm 3.9***	171 \pm 6.3***
		10	134 \pm 4.8***	153 \pm 5.6***
		20	138 \pm 5.1***	109 \pm 5.5
<i>Palmaria palmata</i>	0.19	1	103 \pm 4.2	140 \pm 10.1***
		10	116 \pm 4.9*	127 \pm 6.4**
		20	102 \pm 4.2	91 \pm 4.6

Activities represent the values relative to the vehicle control ($n = 60\text{--}80$ neurons for each seaweed extract)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. 1 Optimal EKA concentration for neurite-outgrowth-promoting activity. **a** Representative phase-contrast photomicrographs of EKA ($1 \mu\text{g mL}^{-1}$) and vehicle. **b** EKA concentration optimization for NPN (triangle), TLPN (square), and LLN (circle) of hippocampal neurons after 2 days of incubation. *V* indicates vehicle. Different letters (*a–d*) indicate significant differences among the means ($P < 0.05$). Data represent means \pm SE ($n = 60\text{--}80$ neurons for each concentration)



bipolar (two processes), and multipolar (more than two processes, Fig. 3a). Of the neuronal cells cultured in EKA-containing medium for 24 h, 48 % developed multipolar characteristics compared with 21 % in the control culture (Fig. 3b, (1)). After 48 h, only 9 % of the total cells in EKA medium did not exhibit bipolar characteristics, whereas 28 % of the cells in the vehicle medium remained unipolar (Fig. 3b, (2)). Thus, the number of processes in EKA-treated cultures was significantly increased compared with that in the vehicle control at both 24 and 48 h ($P < 0.05$). These results clearly indicate that EKA not only enhances neuronal maturation but also accelerates their initial differentiation.

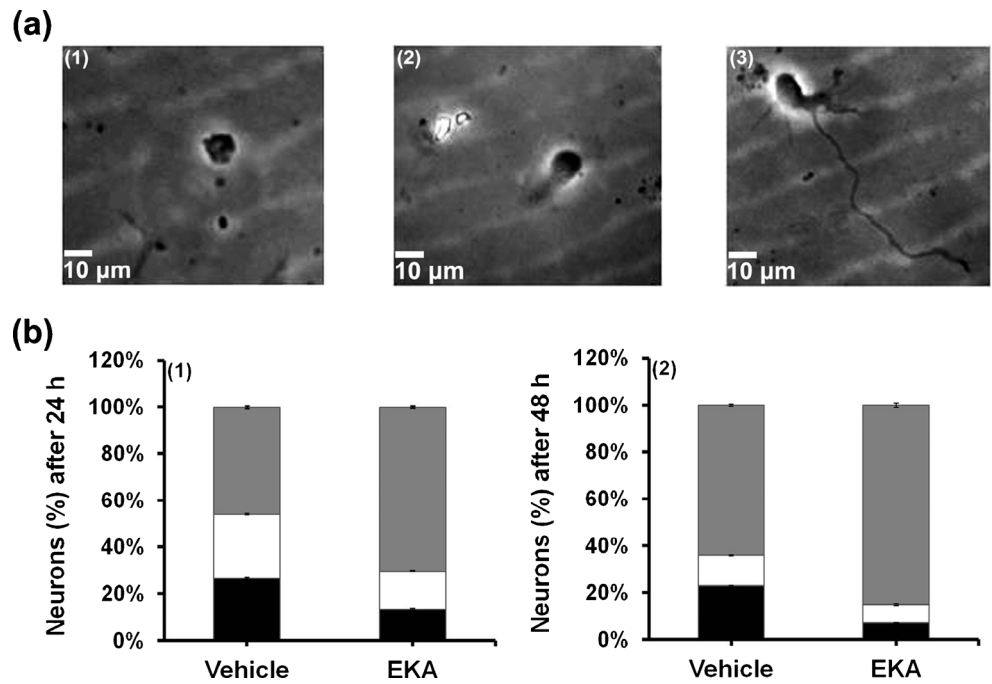
Discussion

We compared the effects of common tropical seaweeds on the neurite outgrowth of primary hippocampal neurons. Among the 34 seaweed species tested, most rhodophytes enhanced neurite outgrowth based on morphological parameters. Most agar or carrageenan producers also demonstrated NOPAs. Few phaeophytes possessed neurotrophic factors, and many inhibited neurite outgrowth, likely due to their phenolic or tannin components. It will be also important to identify NOPA inhibitors to warn humans about consuming food that

contains these substances. Our data suggest that *K. alvarezii* (also commercially known as cottonii) exhibited the greatest enhancement of neuronal differentiation. Approximately 1.5 million tonnes of dry cottonii was harvested from aquaculture in Indonesia in 2009 (Pambudi et al. 2010). The effect of EKA on initial neuronal development was evidenced by the early development of multipolar characteristics. Neuron cells that develop neurites earlier are thought to develop more extensive arborization. In our previous study (Hannan et al. 2013), the red seaweed *Gelidium amansii* exhibited the greatest NOPA. At DIV 3, its ethanol extract showed increased NPN (133 %), TLPN (186 %), and LLN (211 %) values; however, *K. alvarezii* at DIV 2 showed increased NPN (137 %), TLPN (159 %), and LLN (144 %) values. The optimal dose of EKA ($1 \mu\text{g mL}^{-1}$) was markedly lower than that of *Gelidium amansii* ($15 \mu\text{g mL}^{-1}$) for NOPA. Thus, *K. alvarezii* was assumed to have potent neurotrophic compounds. This carrageenan producer *K. alvarezii* also exhibited antioxidant (Kumar et al. 2008), cardiovascular protective (Matanjan et al. 2010), antimicrobial (Prabha et al. 2013), and anti-inflammatory (Ranganayaki et al. 2014) activities.

NF levels in the brain affect neuronal development and survival; thus, NFs are required to prevent neuronal aging and neurodegenerative symptoms, such as Alzheimer's disease (Heese et al. 2006). The Chinese medicinal herb

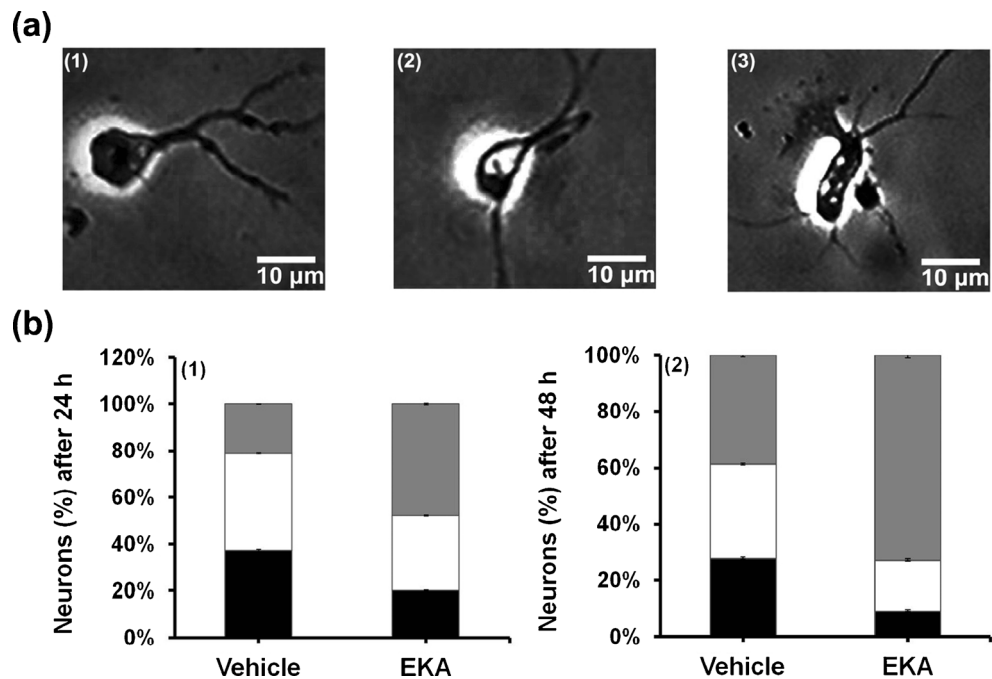
Fig. 2 Effects of EKA on neuronal development. **a** Phase-contrast photomicrographs showing initial differentiation of developing neurons: stage I (lamellipodia, 1), stage 1.5 (transition stage, fan-shaped process, 2), and stage II (minor process, 3). **b** Relative ratio of neurons (%) in the various developmental stages of stage 1 (black bar), stage 1.5 (white bar), and stage 2 (gray bar) after 24 h (1) and 48 h (2) of incubation. Values are percentages of 1000–1200 neurons



Achyranthes bidentata has been reported to prevent the apoptosis and enhance the survival and growth of rat hippocampal neurons in primary cultures (Tang et al. 2008). Ginkgolide B from *Ginkgo biloba* increased brain-derived NFs and protected hippocampal neurons from apoptosis (Xiao et al. 2010). The brown seaweed *S. macrocarpum* exhibits NOPA and supports the survival of PC12D cells due to production of sargaquinoic acid (Kamei and Tsang 2003) and sargachromenol (Tsang et al. 2005). *Sargassum fulvellum* also promoted maturation, synaptogenesis, and survival of

hippocampal neuron cells (Hannan et al. 2012). The active compound from *S. fulvellum* responsible for the differentiation of PC12D cells was pheophytin a (Ina et al. 2007). Phlorotannins from *Eisenia bicyclis* have been shown to inhibit β -amyloid cleavage enzyme (Jung et al. 2010), which initiates the formation of β -amyloid in Alzheimer’s disease (Tang et al. 2006). The ethanol extract of *K. alvarezii* contains alkaloids, carbohydrates, glycosides, proteins, amino acids, phenolic compounds, flavonoids, and terpenoids (Prabha et al. 2013). The aqueous extract had considerable quantities

Fig. 3 Effects of EKA on the processes of neuronal development. **a** Typical phase-contrast images of neurons with unipolar (1), bipolar (2), and multipolar (3) characteristics. **b** Relative ratios of neurons (%) that exhibited unipolar (black bar), bipolar (white bar), and multipolar (gray bar) characteristics after 24 h (1) and 48 h (2) of incubation. Values are percentages of 500–800 neurons



of alkaloids, saponin, phenols, proteins, phytosterols, amino acids, sugars, reducing sugars, flavonoids, steroids, and tannins (Ranganayaki et al. 2014). The major active compound for NOPAs from EKA has not been determined. Our data suggest that the abundant, aquaculturable, and edible *K. alvarezii* may exert beneficial effects on the health of the human brain. Efforts to isolate the active compounds and determine the underlying molecular mechanism(s) are in process.

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Compliance with ethical standards All animal care and use were in accordance with the institutional guidelines and approved by the Institutional Animal Care and Use Committee of the College of Medicine, Dongguk University, South Korea

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