ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 32 (2011) 325–335

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/etap

Review

Potential pharmacological applications of polyphenolic derivatives from marine brown algae

Noel Vinay Thomas[a], Se-Kwon Kim[a,b,*]

[a] Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea
[b] Marine Bioprocess Research Center, Pukyong National University, Busan 608-737, Republic of Korea

ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form
24 August 2011
Accepted 2 September 2011
Available online 17 September 2011

Keywords:
Laminariaceae
Brown algae
Phlorotannins
Bioactive compounds

ABSTRACT

Recently, the isolation and characterization of the biologically active components from seaweeds have gained much attention from various research groups across the world. The marine algae have been studied for biologically active components and phlorotannins are one among them. Among marine algae, brown algal species such as *Ecklonia cava*, *Eisenia arborea*, *Ecklonia stolinifera* and *Eisenia bicyclis* have been studied for their potential biological activities. Majority of the investigations on phlorotannins derived from brown algae have exhibited their potentiality as antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, anti-allergic, hyaluronidase enzyme inhibition and in matrix metalloproteinases (MMPs) inhibition activity. In this review, we have made an attempt to discuss the potential biological activities of phlorotannins from marine brown algae and their possible candidature in the pharmaceutical applications.

Contents

* *Corresponding author at*: Marine Biochemistry Laboratory, Department of Chemistry, Pukyong National University, Busan 608-737, Republic of Korea. Tel.: +82 51 629 7094; fax: +82 51 629 7099.
E-mail address: sknkim@pknu.ac.kr (S.-K. Kim).
1382-6689/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2011.09.004

1. Introduction

In Asian countries such as Korea, Japan and China, marine algae are considered as sea vegetables in the diet and also as an alternative medicine since ancient times (Ali et al., 2000). Recent epidemiological and clinical studies have specified that consumption of plant derived foods and drinks for instance tea, red wine and soya bean products could reduce the risk of oxidative damage related diseases like aging and other lifestyle diseases (Shibata et al., 2008). The health preservative aspects for these natural foods are thought to be attributed because of the presence of polyphenols with antioxidant activity, and many active substances (e.g., catechins, resveratrol and isoflavones) (Aggarwal and Shishodia, 2006). The polyphenols are common secondary metabolites found universally in all plants (Haslam and Cai, 1994). Although terrestrial polyphenols, flavonoids and gallic acids are known to have several bioactive functions (Hollman and Katan, 1999), literature on marine algal polyphenols from a human physiological viewpoint is very sparse (Shibata et al., 2008). Latest studies have focused on the role of dietary factors such as phenolic compounds or polyphenols in the prevention of significant diseases including cancer, coronary heart diseases and allergies (Shibata et al., 2003).

Marine brown algae *Ecklonia cava* and *Eisenia bicyclis* have been investigated for its human beneficial bioactive components including phlorotannins, polysaccharides such as alginic acid, fucoidans, pyropheophytin, tripeptides and oxylipin and also the beneficial bioactivities that include anti-inflammation, inhibition of hyaluronidase activity and anti-diabetic activity (Kojima et al., 1993; Kousaka et al., 2003; Okada et al., 2004; Shibata et al., 2002; Whitaker and Carlson, 1975). A summary of marine algal phlorotannins and their possible pharmacological activities is shown in Table 1. Phlorotannins are suggested to be formed biosynthetically via acetate–malonate pathway, also known as polyketide pathway (Arnold and Targett, 2002). The phlorotannins are highly hydrophilic components with a wide range of molecular sizes ranging between 126 kDa and 650 kDa (Ragan and Glombitza, 1986). Phlorotannins are tannin derivatives composed of several phloroglucinol units linked to each other in different ways and mostly isolated from brown algae and the chemical structures of few marine algal phlorotannins are shown in Fig. 1 (Singh and Bharate, 2006). The members of Laminariaceae are reported to be the rich resources of phlorotannins among other marine algae (Okada et al., 2004). The well studied phlorotannins from *E. bicyclis* and *E. cava* are phloroglucinol, phloroglucinol tetramer, eckol, phlorofucofuroeckol A, dieckol, 8,8′-bieckol, dioxinodehydroeckol (Jung et al., 2010; Li et al., 2009; Shibata et al., 2004). Moreover, few other novel phlorotannins from other edible sea weeds have been reported such as phlorofucofuroeckol A, triphloroethol B, 2-phloroeckol, 7-phloroeckol, diphloroethol, fucofuroeckol

Table 1 – Summary of sea-weed derived phlorotannins and their possible pharmacological applications.

Pharmacological Application	Phlorotannin	Marine algae	References
Anti-diabetic	Dieckol	*E. bicyclis* and *E. cava*	Okada et al. (2004) and Lee et al. (2009)
	Eckol	*E. bicyclis*	Okada et al. (2004)
	Phlorofucofuroeckol-A	*E. stolonifera*	Jung et al. (2008)
	Diphlorethohydroxycarmalol	*I. okamurae*	Heo et al. (2009a)
Anti-cancer	Dioxinodehydroeckol	*E. cava*	Kong et al. (2009)
Anti-hypertension	Eckol		
	Phlorofucofuroeckol A	*E. stolonifera*	Jung et al. (2006)
	Dieckol		
Anti-photoaging	Dieckol		
	Eckol	*E. cava* and *E. stolonifera*	Heo et al. (2009b) and Joe et al. (2006)
MMP inhibition	Dieckol	*E. cava*	Ryu et al. (2009a)
	Eckol and Fucofuroeckol-A	*E. bicyclis*	Lee (2010)
Anti-oxidation	8,8′-Bieckol		
	Phlorofucofuroeckol A		
	Eckol	*E. bicyclis*, *E. cava* and *E. kurome*	Shibata et al. (2008)
	Dieckol		
Anti-HIV	6,6′-Bieckol		
	8,8′-Bieckol	*E. cava*	Artan et al. (2008) and Ahn et al. (2004)
	8,8′-Dieckol		
Anti-allersy	6,6′-Bieckol	*E. cava*	Le et al. (2009)
	Phlorofucofuroeckol B	*E. arborea*	Sugiura et al. (2006)
	Eckol and dieckol	*E. stolonifera*	Joe et al. (2006)

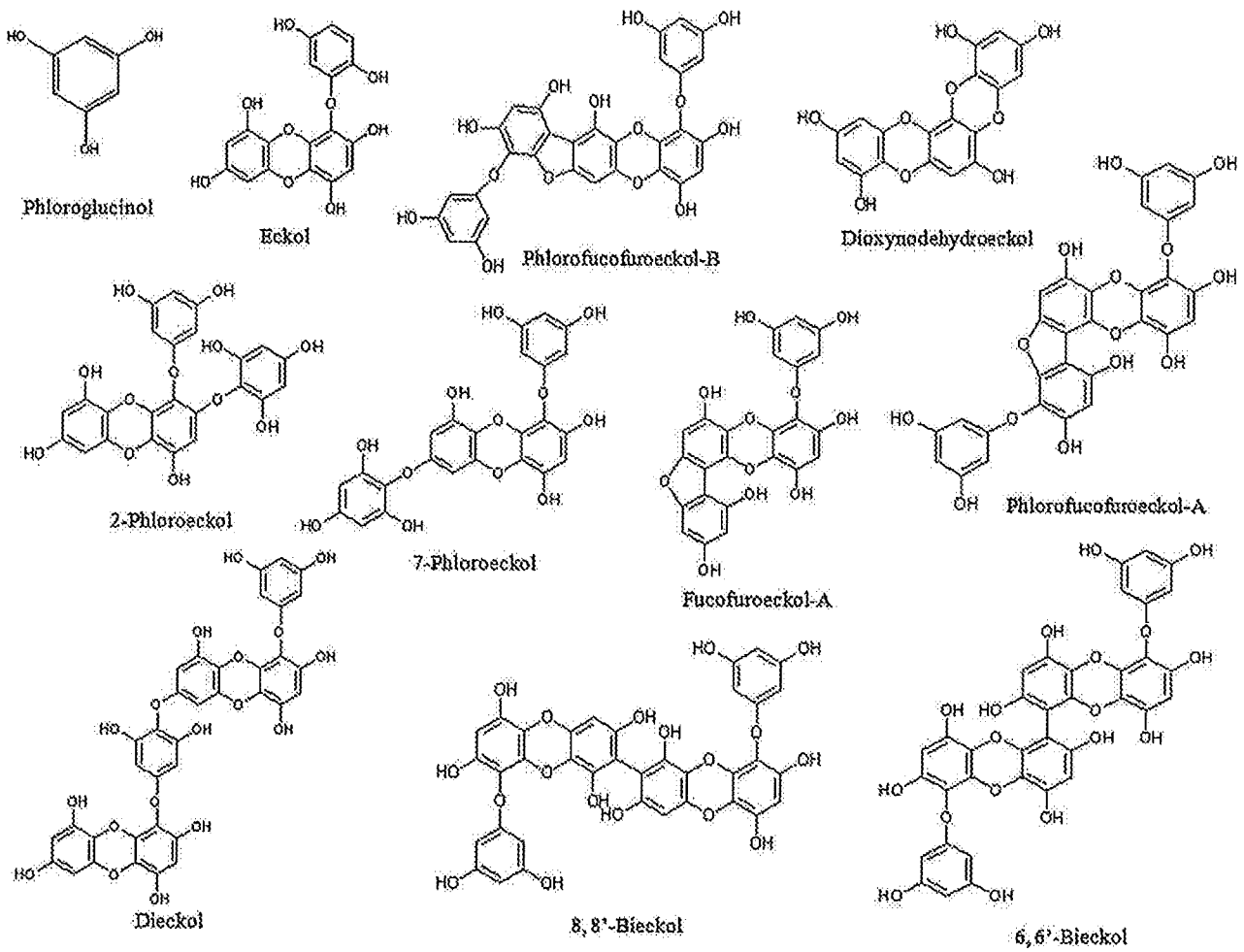

Fig. 1 – Chemical structures of phlorotannins from marine brown algae.

A from *Ecklonia stolinifera* and phlorofucofuroeckol A from *Ecklonia kurome* (Wijesekara et al., 2010). In this review we have made an attempt to narrate the health beneficial activities of brown algal phlorotannins and their possible potential role in pharmaceutical, food and cosmeceutical industries.

2. Potential pharmacological applications of marine brown algal phlorotannins

2.1. *Anti-diabetic activity*

Diabetes mellitus (DM) is one of the most expensive and troublesome chronic diseases and is recognized as a condition that is increasing in the epidemic proportions throughout the world (King et al., 1998). According to the World Health Organization's 2009 report (WHO, 2010a), it is believed that more than 220 million people worldwide have DM and the treatment for diabetes without any side effects is still a challenge in the medical research (Chakrabarti and Rajagopalan, 2002). In medical terms, DM is considered as a chronic disease that is caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn could damage many of the body's systems, including blood vessels and nerves (Matsui et al., 2007). DM is classified into two types, type I and type II. While type I diabetes can be effectively controlled by the administration of insulin, but it is difficult to find an effective treatment for type II diabetes, due to its noninsulin-dependent nature, that contributes for more than 90% of diabetic population (Apostolidis and Lee, 2010). The enzymes α-glucosidase and α-amylase play a significant role in the digestion of dietary complex carbohydrates. Inhibition of these two enzymes can not only slowdown the digestion of oligosaccharides and disaccharides but also delays glucose absorption as well as reduction of glucose levels in plasma, that ultimately leads to the suppression of postprandial hyperglycemia (Lebovitz, 1997). To control glycemic responses in DM type II patients, synthetic glucosidase inhibitor drugs like acarbose (glucobay), miglitol are used (van de Laar et al., 2005). But increasing efforts are being considered to explore and discover potential α-glucosidase and α-amylase inhibitors from natural resources (Bhandari et al., 2008; Welsch et al., 1989). The inhibitory effects of phloroglucinol derivatives from *E. bicyclis* on the glycation and α-amylase have been reported by one of the research groups. They have isolated one novel (compound 1) and two known phloroglucinol derivatives (compound 2 and compound 3). Compound

1 which had maximum similarities with eckol but had extra four glucinol rings, compound 2 was confirmed as eckol and compound 3 was confirmed as dieckol. The inhibitory effect on glycation by ELISA technique revealed the percentages of inhibition as 91.1% for compound 1, 96.2% for compound 2 and 86.7% for compound 3 at 1 mM. On the other hand, at 1 mM concentration, percent inhibition for α-amylase was found to be 89.5%, 87.5% and 97.5% for compounds 1, 2 and 3 respectively recommending phloroglucinol derivatives from brown algae exhibit anti-diabetic properties (Okada et al., 2004). Phlorofucofuroeckol-A isolated from *Ecklonia stolonifera* has shown preventive effects on diabetic complications by exhibiting significant inhibitory effects against advanced glycation end products (AGE), Angiotensin converting enzyme (ACE), rat lens aldose reductase (RLAR), peroxynitrite and reactive oxygen species *in vitro*. Thus suggesting phlorotannins derived from *E. stolonifera* as promising natural products for the treatment of diabetic complications (Jung et al., 2008). According to another research team, polyphenol-rich extract from *E. cava* (ECM) is capable of improving the hyperglycemic status in streptozotocin-induced type I diabetes mellitus rats. Their *in vivo* studies suggest that treatment with ECM significantly lowered the plasma glucose level by 66.4% in diabetes mellitus rats. These results hint that ECM could aid in improving the hyperglycemic status of type I diabetes (Kang et al., 2010).

In the search of natural α-glucosidase inhibitors from marine algal sources, methanolic extracts of *E. stolonifera* (MEE) were checked for their antidiabetic activities in genetically diabetic KK-A^y mice. Interestingly, the MEE that are rich in polyphenolic compounds called phlorotannins have successfully suppressed the increase in plasma glucose and lipid peroxidation levels. Therefore it can be suggested that *E. stolonifera* and its bioactive polyphenols have the potentiality to be developed as antidiabetic pharmaceuticals and functional foods (Iwai, 2008). The antidiabetic activities of *E. cava* derived phlorotannin dieckol were evaluated and it was reported that at IC_{50} values of 10.8 μM/l and 124.9 μM/l dieckol has successfully inhibited the rat intestinal α-glucosidase and porcine pancreatic α-amylase, respectively. Moreover, Lineweaver-Burk plots suggested that dieckol had exhibited a non-competitive mode of inhibitory activity on α-glucosidase (Lee et al., 2009). Diphlorethohydroxycarmalol (DPHC), a kind of phlorotannin that was isolated from *Ishige okamurae*, a brown algae has shown inhibitory effect against α-glucosidase and α-amylase. The IC_{50} values of DPHC against α-glucosidase and α-amylase were 0.16 mM and 0.53 mM, respectively, which were considered to be effective than that of acarbose, a commercial carbohydrate digestive enzyme inhibitor. Further, DPHC may delay the absorption of dietary carbohydrates in the intestine, leading to suppression of an increased blood glucose level after a meal. Thus suggesting DPHC as a potential therapeutic candidate for treating diabetes (Heo et al., 2009a).

2.2. Anti-cancer activity

Phlorotannins possess anticarcinogenic effects (Harada and Kamei, 1997). Phlorotannin extract (PE) derived from brown algae *Laminaria japonica* has shown considerable antiproliferative activity in the human hepatocellular carcinoma cell line (BEL-7402) and on murine leukemic cell line (P388) in a dose dependant manner. The half-inhibitory concentration of PE (IC_{50}) on P388 and BEL-7402 cells was 120 μg/ml and >200 μg/ml, respectively. Microscopic observations have revealed that the morphologic features of tumor cells treated with PE and 5-fluorouracil (a commercial chemotherapy drug) are markedly different from the normal control group suggesting the anti-proliferative effect of PE (Yang et al., 2010). Dioxinodehydroeckol isolated from *E. cava* has exhibited a remarkable anti-proliferative effect on human breast cancer cells (MCF-7). Dioxinodehydroeckol inhibited the proliferation of MCF-7 cells with rates of approximately 25%, 40%, 53%, 56% and 64% at concentrations of 1, 5, 10, 50 and 100 μM, respectively, compared to the control group. *In vitro* studies suggests that dioxinodehydroeckol's potential anti-proliferative activity might be associated with the induction of apoptosis through nuclear factor kappa-light-chain-enhance of activated B cells (NF-κB) family and NF-κB dependent pathway (Kong et al., 2009). The enzymatic extract of *E. cava* together with its crude polysaccharide (CpoF) and crude polyphenolic fractions (CphF) have been reported to possess antiproliferative and antiradical activities. Especially the CphF at an IC_{50} value 5.1 μg/ml has inhibited cell proliferation in murine colon cancer cell line (CT-26). The anti-proliferative effect of CphF is believed to be associated with apoptotic cell demise in CT-26 confirmed by the nuclear staining experiment (Athukorala et al., 2006). The anti-proliferative activity depends on the total polyphenolic content in the algae. For example, the antiproliferative effects of red alga, *Palmaria palmate* and three kelp *Laminaria setchellii*, *Macrocystis integrifolia*, *Nereocystis leutkeana* extracts has been studied on human cervical adenocarcinoma cell line (HeLa cells). HeLa cell proliferation was inhibited between 0% and 78% by *P. palmate*; 0% and 55% by *L. setchellii* and 0% and 69% by *M. integrifolia* and *N. leutkeana* at 0.5–5 μg/ml algal extract. This investigation proves the effectiveness of polyphenolic compounds in controlling tumor growth and brings front a fact that marine algae could serve beneficial for anti-cancer properties (Yuan and Walsh, 2006). In pre-tumor bearing mouse, the dietary feeding of brown algae polyphenols at the rate of 0.1% and 0.5% has significantly reduced tumor multiplicity by 45% and 56% and tumor volume by 54% and 65%, and the topical administration at 3 and 6 mg has significantly decreased tumor multiplicity by 60% and 46% and tumor volume by 66% and 57%, respectively. It is believed that brown algal polyphenols inhibit the cyclooxygenase-2 activity and cell proliferation hence preventing the tumor progression (Hwang et al., 2006).

2.3. ACE inhibition activity

Increased blood pressure or hypertension is one of the chronic medical conditions that aids for the progress of cardiovascular diseases, stroke and the end stage of renal disease (Zhang et al., 2006). Angiotensin I converting enzyme (ACE) plays a significant physiological role in regulating blood pressure (Iroyukifujita et al., 2000). ACE (peptidyl carboxy peptidase, EC 3.4.15.1) belongs to the class of zinc proteases that needs zinc and chloride for activation. ACE converts the inactive form of the decapeptide, angiotensin I, to potent vasopressor octapeptide, angiotensin II. Consequently, the

best therapeutic approach to control hypertension is to inhibit the ACE activity (Lee et al., 2010). The unwanted side-effects of synthetic ACE inhibitors have diverted the focus of researchers on ACE inhibitors derived from functional foods and natural bio-resources (Actis-Goretta et al., 2006; Atkinson and Robertson, 1979).

In the course of exploration for the potential ACE inhibitors from natural sources, six phlorotannins and a fucosterol obtained from *E. stolonifera* have been studied for their ACE inhibitory effects. Among the six isolated phlorotannins eckol, phlorofucofuroeckol A and dieckol have shown a marked ACE inhibitory activity with IC_{50} values of 70.82 ± 0.25, 12.74 ± 0.15, and $34.25 \pm 3.56\,\mu M$, respectively (Jung et al., 2006). It is believed that polyphenolic compounds inhibit ACE activity through sequestration of the enzyme metal factor, Zn^{2+} ion (Liu et al., 2008). Therefore, it can be assumed that phlorotannins might form a complex with the proteins or glycoproteins that are involved in the inhibition of ACE activity. The enzymatic digest of *E. cava* by flavourzyme is reported to possess high capacity of phlorotannins that were checked for potential ACE inhibition in comparison with the commercial ACE inhibitor captopril. The IC_{50} value of flavourzyme enzymatic digest of *E. cava* was reported to be $0.3\,\mu g/ml$ where as for captopril it was $0.05\,\mu g/ml$, suggesting high phlorotannin content gives better ACE inhibition (Athukorala and Jeon, 2005). Apart from brown algae, metabolites from marine red algae are being considered as potential ACE inhibitors. Another research group has worked on the aqueous extracts of red algae *Lomentaria catenata* and *Lithophyllum okamurae*. Their observations have reported that aqueous extracts of these red algae at 20 °C exhibited potent ACE inhibitory activity with IC_{50} values of $12.21\,\mu g/ml$ and $13.78\,\mu g/ml$, respectively. The methanolic extracts from *Ahnfeltiopsis flabelliformis* that were obtained at 20 °C possessed the highest ACE inhibitory activity at IC_{50} value of $13.8\,\mu g/ml$ (Cha et al., 2006). As per the above discussed reports, it is evident that phlorotannins from marine algae could serve as a source for ACE inhibitory substances. It is also recommended that the screening of phlorotannins from other members of marine algae as a comparative study to those that are derived from brown algae could aid for establishing a wide choice for natural ACE inhibitory substances.

2.4. *Photoaging prevention activity*

Human skin is considered as a potential anatomical barrier that acts as an important screen between internal and external environment and supports bodily defense against pathogens and damage (Pallela et al., 2010). Continuous exposure to ultra violet (UV) radiations leads to numerous complications that are correlated with various pathological consequences of the skin damage and when the exposure to UV light exceeds the protective capacity of an individual's melanin is affected and sunburn occurs (Agar et al., 2004; Ryu et al., 2009b). Cosmetics are commercially available products that are used to improve the appearance of the skin. In recent years, the number of women concerning about whiter skin complexion has increased dramatically, especially in Asian continent (Tengamnuay et al., 2006). When skin is repeatedly exposed to UV rays, it undergoes oxidative stress that ultimately manifests as erythema (Thiele et al., 1997). UV radiations have a strong oxidative component, and photo-oxidative stress has been directly linked to the onset of skin photodamage (Caddeo et al., 2008; Fuchs, 1998). Hence it is recommended that regular intake of dietary antioxidants (Messina et al., 1994) or treatment of the skin with products containing antioxidant ingredients might be useful in preventing UV-induced skin damages. Further, it is known that marine brown algae contain phenolic compounds with antioxidant activity and the phlorotannins show an ability to absorb UV light (Henry and Van Alstyne, 2004; Pavia et al., 1997; Ragan and Glombitza, 1986).

UV-B radiations are known to induce photo-oxidative stress (Xie et al., 2009). Phlorotannins isolated from *E. cava* have been screened for their ability to inhibit melanogenesis as well as the protective effect against photo-oxidative stress induced by UV-B radiation. Among the other phlorotannins, dieckol was found to have exceptional protective activity against photo-oxidative stress. Out of those three phlorotannins, dieckol effectively decreased the generated reactive oxygen species (ROS) which was recorded as 100.7% at 250 μM. Phloroglucinol and eckol exhibited slightly lower activity against UV-B radiation-mediated ROS levels as 192.4% and 174.4%, respectively at 250 μM. The *in vitro* studies revealed that dieckol exhibited 57.8% protective properties against UV-B radiation-induced DNA damage at a concentration of 50 μM (Heo et al., 2009b). The phlorotannins eckol and dieckol isolated from *E. stolonifera* have attenuated the expression of matrix metalloproteinase -1 (MMP-1) expression in human dermal fibroblasts. These findings reveal that the inhibition of MMP-1 (which is an interstitial collagenase, is mainly responsible for the degradation of dermal collagen in human skin aging process) expression by *E. stolonifera* derived phlorotannins was in correlation with the inhibition of both NF-κB and activator protein-1 (AP-1) reporter activity (Joe et al., 2006). Thus suggesting eckol and dieckol as potential agents to prevent and treat skin aging. A schematic representation of the role of phlorotannins in controlling photoaging is shown in Fig. 2. The effectiveness of brown algal polyphenols in inhibiting UVB-induced skin carcinogenesis in SKH-1 hairless mouse skin model was evaluated. Their *in vivo* reports demonstrated that both dietary feeding and topical treatment of brown algal polyphenols has suppressed cyclooxygenase-2 (COX-2) expression and cell proliferation (Hwang et al., 2006). These results suggest the role of brown algae polyphenols, phlorotannins as potential cancer chemopreventive agents against photocarcinogenesis and other adverse effects of UVB exposure.

2.5. *Matrix metalloproteinase inhibition activity*

Matrix metalloproteinases (MMPs) are a class of structurally similar enzymes that contribute for the extracellular matrix degradation and play a major role in normal and pathological tissue remodeling. Imbalance in the expression of MMPs leads to severe pathological condition that could instigate the risk of cardiac, cartilage, cancer related diseases. Based on substrate specificity, the MMPs are categorized into three major functional groups. The main three groups include interstitial collagenases that have affinities toward collagen types I, II, and III (MMP-1, -8, and -13), the stromelysins with specificity

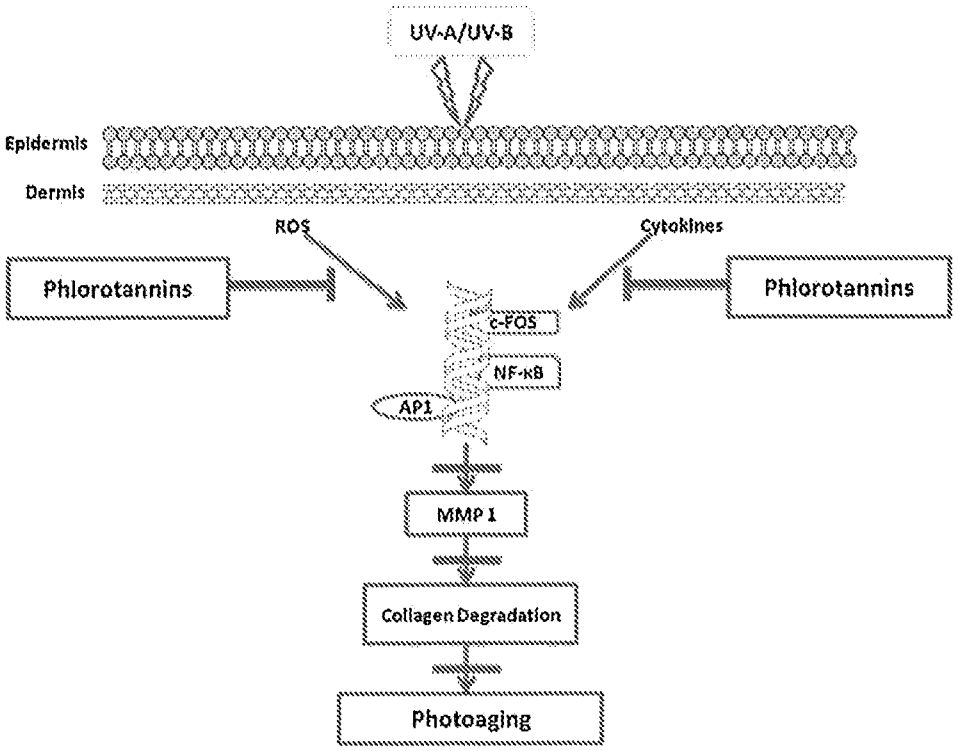

Fig. 2 – Marine algal phlorotannins inhibit the ROS and cytokine production which inturn reduces the expression of MMP-1 and prevents the photoaging.

for laminin, fibronectin, and proteoglycans (MMP-3, -10, and -11), and the gelatinases that effectively cleave type IV and V collagen (MMP-2 and -9) (Nelson et al., 2000; Rajapakse et al., 2007). Commercially successful matrix metalloproteinases inhibitors (MMPIs) like Batimastat (BB-94), Marimastat (BB-516) are known to have strong Zn^{2+} chelating group, hydroxamate. The poor selectivity, improper metabolism, low oral bioavailability, poor solubility, side effects and the risk of increased drug toxicity have strongly eliminated the few synthetic MMPIs from clinical trials (Coussens et al., 2002; Pavlaki and Zucker, 2003; Zhang and Kim, 2009). Until now, several research groups have reported natural MMPIs terrestrial sources (Ha et al., 2004; Seo et al., 2005). For instance, the *in vivo* studies suggest that oral administration of green tea polyphenols (GTPs) has reduced UVB-induced tumor incidence by 35%, tumor multiplicity by 63%, and tumor growth by 55% in UV-B irradiated mice. This study reveals that GTPs reduced the expression of MMP-2 and MMP-9 which have crucial roles in tumor growth and metastasis (Mantena et al., 2005). However, the diversity in marine life forms offers a wide scope to screen biologically active compounds that could be beneficial in designing efficient MMPIs (Jain et al., 2009; Noel Vinay and Se-Kwon, 2010). The inhibition of MMP activity by marine derived chitooligosaccharides, flavonoids, polyphenols, and fatty acids has been previously reviewed (Zhang and Kim, 2009). Dieckol and 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6″-trihydroxyphenoxy) 2,4,9-trihydroxydibenzo-1,4-dioxin from the methanolic extract of *E. cava* has remarkably promoted osteosarcoma differentiation by elevating the alkaline phosphatase activity, mineralization, total protein and collagen synthesis in human osteosarcoma cells (MG-63). Moreover the inhibition of MMP-1, MMP-3, and MMP-13 by these phlorotannins at gene and protein levels was confirmed by casein zymography, western blot and reverse transcriptase-polymerase chain reaction (RT-PCR) assays (Ryu et al., 2009a). A detailed *in vitro* study on the inhibitory effects of phlorotannins derived from *E. cava* on MMPs activities has revealed that at 20 μg/ml, these phlorotannins have inhibited the bacterial collagenase-1 activity suggesting the phlorotannins efficacy to inhibit collagenases that could aid in MMPIs development (Kim et al., 2006). *E. bicyclis* derived phlorotannins fucofuroeckol-A and eckol inhibited the expression of MMP-2 and MMP-9 in human fibrosarcoma cell line HT1080. It was reported that fucofuroeckol-A and eckol inhibited the NF-κB expression and also had a significant inhibitory effect on activator protein-1 (AP-1) expression. Thus inhibiting the expression of MMP-2 and -9 via blocking the transcription of both NF-κB and (AP-1) (Lee, 2010). Therefore, marine brown algal members could serve as potential sources for the development of pharmaceuticals against MMPs and cancer.

2.6. *Reactive oxygen species scavenging activity*

Reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (HO•) and hydrogen peroxide (H_2O_2) formed during aerobic life. DNA, cell membranes, proteins and other cellular constituents are the target sites of the degradation processes, and could induce atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as

well as aging (Ruberto et al., 2001). As a protection for cellular biomolecules, equilibrium between oxidants formation and endogenous antioxidant defense mechanisms exist. If this balance is disturbed, it can produce oxidative stress. Some synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone, and propyl gallate used in food industry have been identified as mutagens (Kahl, 1984; Kahl and Kappus, 1993). The proteolytic hydrolysates from *E. cava* were investigated for its radical scavenging effect and inhibition of H_2O_2 induced DNA damage. All the proteolytic hydrolysates exhibited strong dose-dependent radical scavenging activities (above 80%) at a concentration of 2.5 μg/ml (Heo et al., 2005). Similarly, another research group has reported the potential antioxidant activities of three phlorotannins (phloroglucinol, eckol and dieckol) purified from *E. cava*. Their results suggest that eckol samples scavenged around 93% of DPPH at 0.25, 0.5, 1 mg/ml of concentrations and were higher than the other phlorotannins. Moreover protective effects of the phlorotannins against H_2O_2 mediated DNA damage increased with increased concentrations of the samples in the mouse T-cell lymphoma cell line (L5178Y-R) (Ahn et al., 2007). In the evaluation of radical scavenging capability of the enzymatic extracts from *I. okamurae*, it has been reported that all of the enzymatic extracts showed profound dose-dependent radical scavenging abilities. The enzymatic digests of the five proteases, alcalase, flavourzyme, kojizyme, neutrase and protamex had shown DPPH scavenging activities of 94.30, 81.70, 66.85, 73.70, and 83.72%, respectively. In particular, the cytoprotective effects of the Kojizyme extract against H_2O_2-induced DNA damage increased significantly with increasing extract concentrations (Heo and Jeon, 2008). Oligomers of phloroglucinol (1,3,5-trihydroxybenzene), eckol (a trimer), phlorofucofuroeckol A (a pentamer), dieckol and 8,8′-bieckol (hexamers), isolated from the Laminarian brown algae *E. bicyclis*, *E. cava* and *E. kurome*, showed potent inhibition of phospholipid peroxidation at 1 μM in the liposome system. The phlorotannins had significant radical scavenging activities against the superoxide anion (50% effective concentration values: 6.5–8.4 μM) and DPPH (50% effective concentration values: 12–26 μM). The EC_{50} values of the phlorotannins eckol, phlorofucofuroeckol A, dieckol and 8,8′-bieckol were 26, 12, 13 and 15 μM, respectively. In contrast, those of catechin, EGCG, ascorbic acid and α-tocopherol were 32, 7.4, 30, and 52 μM, respectively. The phlorotannins were potent free radical scavengers and about twice as effective as catechin, ascorbic acid and α-tocopherol (Shibata et al., 2008).

2.7. *Anti-HIV activity*

Human immunodeficiency virus type-1 (HIV-1) has been recognized as the responsible agent for acquired immunodeficiency syndrome (AIDS) which is one of the most extensively studied diseases and that by the end of 2008 an estimated 33.4 million people worldwide were living with HIV (WHO, 2010b). The first generation anti-HIV drugs have been developed to treat AIDS patients after the introduction of AIDS in early 1980. However, due to the development of drug resistant strains of virus majorly attributed for the failure of anti-AIDS treatment in most of the patients infected with HIV. Therefore, the exploration for potential drug candidates those could render higher inhibitory activity against various HIV strains is increasing in pharmaceutical industry. In this regard, natural bioactive compounds and their derivatives are great sources for the development of new generation anti-HIV therapeutics, which are more effective with minor side-effects (Schaeffer and Krylov, 2000). Having such a big potential, natural product research has increasingly turned to marine natural products, and some of them are currently in clinical or preclinical evaluation. Marine organisms are among the leading sources of anti-HIV natural products. The antiviral properties of cyanovirin-N, an 11-kDa protein from cyanobacteria, were investigated and found that it irreversibly inactivates HIV through its high affinity to glycoprotein 120 (gp120) (De Clercq, 2000; Queiroz et al., 2008). 6,6′-Bieckol, one of the major phloroglucinol derivative that naturally occurs in *E. cava*, has been reported to inhibit HIV-1 induced syncytia formation, lytic effects, and viral p24 antigen production *in vitro* and in cellular experiments. Moreover, at IC_{50} values of 1.07 μM, 6,6′-bieckol has selectively inhibited the activity of HIV-1 reverse transcriptase enzyme and also has prevented the entry of HIV-1. Furthermore, it exhibited no cytotoxicity at concentration, where it has inhibited HIV-1 replication almost completely (Artan et al., 2008). Another research group has reported the inhibitory activity of 8,8′-bieckol and 8,4″-dieckol on HIV-1 reverse transcriptase (RT) and protease. Their investigations revealed that these phlorotannins have inhibited RT more potently than protease. The inhibitory activity of 8,8′-bieckol (IC_{50}, 0.51 μM) against HIV-1 RT could be comparable to that of nevirapine (IC_{50}, 0.28 μM), a commercial drug for HIV-1 treatment (Ahn et al., 2004). These results clearly suggest that phlorotannins can serve as potential inhibitory substances for HIV-1 infections. However, until now *in vitro* anti HIV-1 activities are confined with phlorotannins isolated from *E. cava* species. As phlorotannins are found in the members of brown marine algae, there is a wide scope to come up with more efficient anti-HIV-1 inhibitory substances from other brown algal members.

2.8. *Anti-pruritic inflammatory activity*

One of the most challenging pruritic skin inflammatory diseases that need a better therapeutic approach to be devised is Atopic dermatitis (AD). This skin inflammatory disease can occur at any age and is characterized exclusively by the elevated serum immunoglobulin E (IgE) levels and eosinophilia. Clinically it is understood that AD is the cutaneous manifestation of a systemic disorder that would also promote asthma, food allergy and allergic rhinitis (Novak and Bieber, 2003). Understanding the fact that inhibition of IgE production or reduction in the concentration of IgE could be the best medical practices to control AD, recombinant humanized anti-IgE monoclonal antibody (mAb), omalizumab (a commercial drug) is being considered for the management of AD (Sheinkopf et al., 2008) and it has shown fair results in managing asthma. However, an enormous amount of mAb is required to remove the IgE from AD patients and hence regarded as an expensive approach. This might have made the researchers to screen for bioactive substances from natural resources that could potentially inhibit the production of IgE.

One of the investigations for the screening of anti-allergic polyphenolic substances from marine algae has resulted in the isolation of phlorotannin derivatives 6,6′-bieckol and 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin for the first time from *E. cava*. These derivatives have exhibited potential anti-allergic mechanism by suppression of binding activity between IgE and FcεRI in human basophilic leukemia (KU812) and rat basophilic leukemia (RBL-2H3) cell lines *in vitro* (Le et al., 2009). Their results suggest that phlorotannins could be used as potential leads for the formulation of drugs to treat AD. *In vitro* studies on phlorofucofuroeckol-B isolated from *Eisenia arborea* in rat basophile leukemia (RBL)-2H3 cells have confirmed the anti-allergic property of phlorotannins (Sugiura et al., 2006). The anti-allergic activity of phlorofucofuroeckol-B was compared with that of epigallocatechin gallate and the resultant IC_{50} values obtained were 7.8 μM and 22 μM respectively suggesting the phlorofucofuroeckol-B as a potent anti-inflammatory agent that could be beneficial to treat AD. FcεRI, a high-affinity receptor for IgE, is expressed by basophils and mast cells on the cell surface and they act as effector cells in allergic related reactions. The effects of methanolic extracts from *E. cava* on the expression of FcεRI in human basophilic KU812F cells suggest that there was reduction in the cell surface expression of FcεRI and RT-PCR data has reported that mRNA expression of total cellular FcεRI α-chain was reduced which is essential in eliciting inflammatory responses (Shim et al., 2009). From these results, it is understood that phlorotannins can be useful in the management of AD through the reduction of IgE concentration and their histamine inhibitory activities. On the other hand phlorotannins eckol and dieckol isolated from *E. stolonifera* were reported to inhibit the expression of matrix metalloproteinase-1 (which is an interstitial collagenase, is mainly responsible for the degradation of dermal collagen in human skin aging process expression) in human dermal fibroblasts. The inhibition of MMP-1 was thought to be in correlation with the inhibition of both NF-κB and activator protein-1 (AP-1) reporter activity (Joe et al., 2006).

2.9. *Other biological activities*

Eckstolonol and phlorofucofuroeckol A isolated from active ethyl acetate fraction of *E. stolonifera* Okamura exhibited protective effect against the cytotoxic effects of tacrine (commercial cholinesterase inhibitor), in human hepatoma cell line (Hep G2). The EC_{50} values for eckstolonol and phlorfucofuroeckol 62.0 and 79.2 μg/ml, respectively, where as the EC_{50} values for phloroglucinol, eckol and dieckol were >100 μg/ml (Kim et al., 2005). Thus suggesting marine brown algae derived phlorotannins as possible hepatoprotective agents. In the process of screening novel anti-hyperlipidemic agents, *E. stolonifera* was considered by a research team. In their *in vivo* studies, the ethanolic extracts of *E. stolonifera* and its phlorotannin constituents, eckol and dieckol, were tested for their anti-hyperlipidemic activity. Eckol and dieckol induced a significant reduction in serum triglycerides, total cholesterol levels, as well as in the atherogenic index suggesting their potentiality to be exploited for the prevention of hyperlipidemic atherosclerosis (Yoon et al., 2008b). Hyaluronidase (EC 3.2.1.35) is an enzyme that depolymerizes the polysaccharide, hyaluronic acid in the extra cellular matrix of connective tissue. This enzyme is known to be involved in allergic effects, migration of cancers and inflammation. Phlorotannins such as eckol, phlorofucofuroeckol A, dieckol, and 8,8′-bieckol isolated from *E. bicyclis* and *E. kurome* have shown a stronger inhibition effect against hyaluronidase than well-known inhibitors such as catechin and sodium cromoglycate (Shibata et al., 2002). Dried powder of *E. arborea* has suppressed levels of serum immunoglobulin E (IgE) and histamine in Brown Norway rats which strongly respond to IgE. It is suggested that the anti-allergic effect of *E. arborea* dried powder is by inhibition of IgE and anti-degranulation of chemical mediators via a change in the T helper cell clones (Th1/Th2) balance that are responsible for allergic reactions. This clearly suggests that marine brown algal derivatives can be used as potential anti-allergic agents (Sugiura et al., 2008). A new phlorotannin, phlorofucofuroeckol-B was isolated from the *E. arborea* has been reported to exhibit profound inhibitory effect on histamine release from rat basophile leukemia cells (RBL)-2H3 (Sugiura et al., 2006). In addition, some phlorotannins such as 7-phloroeckol, phlorofucofuroeckol A, and 6,6′-bieckol could be used as potential functional food ingredients or nutraceuticals for the prevention of Alzheimer's disease due to their inhibitory activities against the both enzymes acetylcholinesterase and butyrylcholinesterase *in vitro* (Yoon et al., 2008a, 2009).

3. Conclusions and future prospects

In conclusion, due to the abundant chemical distribution of phlorotannins among marine brown algae, it is suggested for future prospects that much focus on the biological and pharmacological activities of phlorotannins from other brown algal species should be considered. It is recommended to screen phlorotannins from other marine macro algae and evaluate their biological activities as a comparative study. In this review, we have made an attempt to discuss the various biological activities associated with phlorotannins that are confined to *in vitro* and *in vivo*. With the latest advancements in the fields of molecular biology and biochemistry, a sophisticated approach to study the interactions of phlorotannins with human cellular systems could prove beneficial in understanding parameters like bioavailability, molecular interactions of phlorotannins involved in the management of various human diseases. On the other hand, this would broaden the chances of screening more biologically efficient phlorotannins and provide with resourceful drug candidates for pharmacological purposes.

Conflict of interest statement

There is no conflict of interest between the two authors.

Acknowledgements

This study was supported by a grant from Marine Bioprocess Research Center of the Marine Bio 21 Project funded by the Ministry of Land, Transport and Maritime, Republic of Korea.

The authors are thankful to Dr. Jayachandran Venkatesan for redrawing the chemical structures of phlorotannins in Fig. 1.

REFERENCES

Actis-Goretta, L., Ottaviani, J., Fraga, C., 2006. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J. Agric. Food Chem. 54, 229–234.

Agar, N., Halliday, G., Barnetson, R., Ananthaswamy, H., Wheeler, M., Jones, A., 2004. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. PNAS 101, 4954–4959.

Aggarwal, B., Shishodia, S., 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem. Pharmacol. 71, 1397–1421.

Ahn, G.N., Kim, K.N., Cha, S.H., Song, C.B., Lee, J., Heo, M.S., Yeo, I.K., Lee, N.H., Jee, Y.H., Kim, J.S., 2007. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Eur. Food Res. Technol. 1, 71–79.

Ahn, M.J., Yoon, K.D., Min, S.Y., Lee, J.S., Kim, J.H., Kim, T.G., Kim, S.H., Kim, N.G., Huh, H., Kim, J., 2004. Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. Biol. Pharm. Bull. 27, 544–547.

Ali, M., Jahangir, M., Saleem, M., Pervez, M., Hameed, S., Ahmad, V., 2000. Metabolites of marine algae collected from Karachi-coasts of Arabian sea. Nat. Prod. Sci. 6, 61–65.

Apostolidis, E., Lee, C., 2010. *In vitro* potential of ascophyllum nodosum phenolic antioxidant mediated α-glucosidase and α-amylase inhibition. J. Food Sci. 75, 97–102.

Arnold, T.M., Targett, N.M., 2002. Marine tannins: the importance of a mechanistic framework for predicting ecological roles. J. Chem. Ecol. 28, 1919–1934.

Artan, M., Li, Y., Karadeniz, F., Lee, S.H., Kim, M.M., Kim, S.K., 2008. Anti-HIV-1 activity of phloroglucinol derivative, 6,6′-bieckol, from *Ecklonia cava*. Bioorg. Med. Chem. Lett. 16, 7921–7926.

Athukorala, Y., Jeon, Y., 2005. Screening for angiotensin 1-converting enzyme inhibitory activity of *Ecklonia cava*. J. Food Sci. Nutr. 10, 134–139.

Athukorala, Y., Kim, K., Jeon, Y., 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food Chem. Toxicol. 44, 1065–1074.

Atkinson, A., Robertson, J., 1979. Captopril in the treatment of clinical hypertension and cardiac failure. Lancet 2, 836–839.

Bhandari, M., Jong-Anurakkun, N., Hong, G., Kawabata, J., 2008. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw.). Food Chem. 106, 247–252.

Caddeo, C., Teskac, K., Sinico, C., Kristl, J., 2008. Effect of resveratrol incorporated in liposomes on proliferation and UV-B protection of cells. Int. J. Pharm. 363, 183–191.

Cha, S., Lee, K., Jeon, Y., 2006. Screening of extracts from red algae in Jeju for potentials marine angiotensin-I converting enzyme (ACE) inhibitory activity. Algae Inchon. 21, 343–348.

Chakrabarti, R., Rajagopalan, R., 2002. Diabetes and insulin resistance associated disorders: disease and the therapy. Curr. Sci. 83, 1533–1538.

Coussens, L., Fingleton, B., Matrisian, L., 2002. Matrix metalloproteinase inhibitors and cancer – trials and tribulations. Science 295, 2387–2392.

De Clercq, E., 2000. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med. Res. Rev. 20, 323–349.

Fuchs, J., 1998. Potentials and limitations of the natural antioxidants RRR-α-tocopherol, l-ascorbic acid and β-carotene in cutaneous photoprotection1. Free Radic. Biol. Med. 25, 848–873.

Ha, K.T., Kim, J.K., Kang, S.K., Kim, D.W., Lee, Y.C., Kim, H.M., Kim, C.H., 2004. Inhibitory effect of Sihoga-Yonggol-Moryo-Tang on matrix metalloproteinase-2 and -9 activities and invasiveness potential of hepatocellular carcinoma. Pharmacol. Res. 50, 279–285.

Harada, H., Kamei, Y., 1997. Selective cytotoxicity of marine algae extracts to several human leukemic cell lines. Cytotechnology 25, 213–219.

Haslam, E., Cai, Y., 1994. Plant polyphenols (vegetable tannins): gallic acid metabolism. Nat. Prod. Rep. 11, 41–66.

Henry, B.E., Van Alstyne, K.L., 2004. Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. J. Phycol. 40, 527–533.

Heo, S.J., Hwang, J.Y., Choi, J.I., Han, J.S., Kim, H.J., Jeon, Y.J., 2009a. Diphlorethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent α-glucosidase and α-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. Eur. J. Pharmacol. 615, 252–256.

Heo, S.J., Jeon, Y.J., 2008. Radical scavenging capacity and cytoprotective effect of enzymatic digests of *Ishige okamurae*. J. Appl. Phycol. 20, 1087–1095.

Heo, S.J., Ko, S.C., Cha, S.H., Kang, D.H., Park, H.S., Choi, Y.U., Kim, D., Jung, W.K., Jeon, Y.J., 2009b. Effect of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation. Toxicol. In Vitro 23, 1123–1130.

Heo, S.J., Park, P.J., Park, E.J., Cho, S.K., Kim, S.K., Jeon, Y.J., 2005. Antioxidative effect of proteolytic hydrolysates from *Ecklonia cava* on radical scavenging using ESR and H_2O_2-induced DNA damage. Food Sci. Biotechnol. 14, 614–620.

Hollman, P.C.H., Katan, M.B., 1999. Dietary flavonoids: intake, health effects and bioavailability. Food Chem. Toxicol. 37, 937–942.

Hwang, H., Chen, T., Nines, R.G., Shin, H.C., Stoner, G.D., 2006. Photochemoprevention of UVB induced skin carcinogenesis in SKH 1 mice by brown algae polyphenols. Int. J. Cancer 119, 2742–2749.

Iroyukifujita, H., Eiichiyokoyama, K., Yoshikawa, M., 2000. Classification and antihypertensive activity of angiotensin i converting enzyme inhibitory peptides derived from food proteins. J. Food Sci. 65, 564–569.

Iwai, K., 2008. Antidiabetic and antioxidant effects of polyphenols in brown alga *Ecklonia stolonifera* in genetically diabetic KK-A y mice. Plant Foods Hum. Nutr. 63, 163–169.

Jain, R., Sonawane, S., Mandrekar, N., 2009. Marine life: new hope for cancer drugs. Indian J. Caner 46, 243–244.

Joe, M.J., Kim, S.N., Choi, H.Y., Shin, W.S., Park, G.M., Kang, D.W., Kim, Y.K., 2006. The inhibitory effects of eckol and dieckol from *Ecklonia stolonifera* on the expression of matrix metalloproteinase-1 in human dermal fibroblasts. Biol. Pharm. Bull. 29, 1735–1739.

Jung, H.A., Hyun, S.K., Kim, H.R., Choi, J.S., 2006. Angiotensin converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. Fisheries Sci. 72, 1292–1299.

Jung, H.A., Oh, S.H., Choi, J.S., 2010. Molecular docking studies of phlorotannins from *Eisenia bicyclis* with BACE1 inhibitory activity. Bioorg. Med. Chem. Lett. 20, 3211–3215.

Jung, H.A., Yoon, N.Y., Woo, M.H., Choi, J.S., 2008. Inhibitory activities of extracts from several kinds of seaweeds and phlorotannins from the brown alga *Ecklonia stolonifera* on glucose-mediated protein damage and rat lens aldose reductase. Fisheries Sci. 74, 1363–1365.

Kahl, R., 1984. Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical

mutagens and chemical carcinogens. Toxicology 33, 185–228.
Kahl, R., Kappus, H., 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z. Lebensm. Unters. Forsch. 196, 329–338.
Kang, C., Jin, Y.B., Lee, H., Cha, M., Sohn, E., Moon, J., Park, C., Chun, S., Jung, E.S., Hong, J.S., 2010. Brown alga *Ecklonia cava* attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. Food Chem. Toxicol. 48, 509–516.
Kim, M.M., Ta, Q.V., Mendis, E., Rajapakse, N., Jung, W.K., Byun, H.G., Jeon, Y.J., Kim, S.K., 2006. Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. Life Sci. 79, 1436–1443.
Kim, Y.C., An, R.B., Yoon, N.Y., Nam, T.J., Choi, J.S., 2005. Hepatoprotective constituents of the edible brown alga *Ecklonia stolonifera* on tacrine-induced cytotoxicity in hep G2 cells. Arch. Pharm. Res. 28, 1376–1380.
King, H., Aubert, R., Herman, W., 1998. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care 21, 1414–1431.
Kojima, T., Koike, A., Yamamoto, S., Kanemitsu, T., Miwa, M., Kamei, H., Kondo, T., Iwata, T., 1993. Eisenin (L-pyroGlu-L-Gln-L-Ala), a new biological response modifier. J. Immunother. 13, 36–42.
Kong, C.S., Kim, J.A., Yoon, N.Y., Kim, S.K., 2009. Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. Food Chem. Toxicol. 47, 1653–1658.
Kousaka, K., Ogi, N., Akazawa, Y., Fujieda, M., Yamamoto, Y., Takada, Y., Kimura, J., 2003. Novel oxylipin metabolites from the brown alga *Eisenia bicyclis*. J. Nat. Prod. 66, 1318–1323.
Le, Q.T., Li, Y., Qian, Z.J., Kim, M.M., Kim, S.K., 2009. Inhibitory effects of polyphenols isolated from marine alga *Ecklonia cava* on histamine release. Process Biochem. 44, 168–176.
Lebovitz, H.E., 1997. Alpha-glucosidase inhibitors. Endocrinol. Metab. Clin. 26, 539–551.
Lee, S.H., Karadeniz, F., Kim, M.M., Kim, S.K., 2009. α-Glucosidase and α-amylase inhibitory activities of phloroglucinal derivatives from edible marine brown alga, *Ecklonia cava*. J. Sci. Food Agric. 89, 1552–1558.
Lee, S.H., Qian, Z.J., Kim, S.K., 2010. A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. Food Chem. 118, 96–102.
Lee, S.H., 2010. Anti-inflammatory Mechanisms of Phlorotannins Derived from *Eisenia bicyclis* and Their inhibitory Effects on Matrix Metalloproteinases. Pukyong National University, Busan, South Korea.
Li, Y., Qian, Z.J., Ryu, B.M., Lee, S.H., Kim, M.M., Kim, S.K., 2009. Chemical components and its antioxidant properties in vitro: an edible marine brown alga, *Ecklonia cava*. Bioorg. Med. Chem. Lett. 17, 1963–1973.
Liu, C., Li, X., Li, Y., Feng, Y., Zhou, S., Wang, F., 2008. Structural characterisation and antimutagenic activity of a novel polysaccharide isolated from *Sepiella maindroni* ink. Food Chem. 110, 807–813.
Mantena, S., Meeran, S., Elmets, C., Katiyar, S., 2005. Orally administered green tea polyphenols prevent ultraviolet radiation-induced skin cancer in mice through activation of cytotoxic T cells and inhibition of angiogenesis in tumors. J. Nutr. 135, 2871–2877.
Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Tamaya, K., Miyata, Y., Tanaka, K., Matsumoto, K., 2007. α-Glucosidase inhibitory profile of catechins and theaflavins. J. Agric. Food Chem. 55, 99–105.
Messina, M., Persky, V., Setchell, K., Barnes, S., 1994. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr. Cancer 21, 113–131.
Nelson, A., Fingleton, B., Rothenberg, M., Matrisian, L., 2000. Matrix metalloproteinases: biologic activity and clinical implications. J. Clin. Oncol. 18, 1135–1149.
Noel Vinay, T., Se-Kwon, K., 2010. Metalloproteinase inhibitors: status and scope from marine organisms. Biochem. Res. Int. 2010, 10.
Novak, N., Bieber, T., 2003. Allergic and nonallergic forms of atopic diseases. J. Allergy Clin. Immunol. 112, 252–262.
Okada, Y., Ishimaru, A., Suzuki, R., Okuyama, T., 2004. A new phloroglucinol derivative from the brown alga *Eisenia bicyclis*: potential for the effective treatment of diabetic complications. J. Nat. Prod. 67, 103–105.
Pallela, R., Yoon, N.Y., Kim, S.K., 2010. Anti-photoaging and photoprotective compounds derived from marine organisms. Mar. Drugs 8, 1189–1208.
Pavia, H., Cervin, G., Lindgren, A., Aberg, P., 1997. Effects of UV-B radiation and simulated hebivory on phlorotannins in the brown alga *Ascophyllum nodosum*. MEPS 157, 139–146.
Pavlaki, M., Zucker, S., 2003. Matrix metalloproteinase inhibitors (MMPIs): the beginning of phase I or the termination of phase III clinical trials. Cancer Metast. Rev. 22, 177–203.
Queiroz, K.C.S., Medeiros, V.P., Queiroz, L.S., Abreu, L.R.D., Rocha, H.A.O., Ferreira, C.V., Jucá, M.B., Aoyama, H., Leite, E.L., 2008. Inhibition of reverse transcriptase activity of HIV by polysaccharides of brown algae. Biomed. Pharmacother. 62, 303–307.
Ragan, M.A., Glombitza, K.W., 1986. Phlorotannins, brown algal polyphenols. In: Hellebust, J.A., Craigie, J.S. (Eds.), Progress in Phycological Research. Cambridge University Press, Newyork, pp. 129–241.
Rajapakse, N., Mendis, E., Kim, M.M., Kim, S.K., 2007. Sulfated glucosamine inhibits MMP-2 and MMP-9 expressions in human fibrosarcoma cells. Bioorg. Med. Chem. 15, 4891–4896.
Ruberto, G., Baratta, M.T., Biondi, D.M., Amico, V., 2001. Antioxidant activity of extracts of the marine algal genus Cystoseira in a micellar model system. J. Appl. Phycol. 13, 403–407.
Ryu, B.M., Li, Y., Qian, Z.J., Kim, M.M., Kim, S.K., 2009a. Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: implication for chronic articular disease. Chem. Biol. Interact. 179, 192–201.
Ryu, B.M., Qian, Z.J., Kim, M.M., Nam, K.W., Kim, S.K., 2009b. Anti-photoaging activity and inhibition of matrix metalloproteinase (MMP) by marine red alga, *Corallina pilulifera* methanol extract. Radiat. Phys. Chem. 78, 98–105.
Schaeffer, D.J., Krylov, V.S., 2000. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol. Environ. Saf. 45, 208–227.
Seo, U.K., Lee, Y.J., Kim, J.K., Cha, B.Y., Kim, D.W., Nam, K.S., Kim, C.H., 2005. Large-scale and effective screening of Korean medicinal plants for inhibitory activity on matrix metalloproteinase-9. J. Ethnopharmacol. 97, 101–106.
Sheinkopf, L.E., Rafi, A.W., Do, L.A.T., Katz, R.M., Klaustermeyer, W.B., 2008. Efficacy of omalizumab in the treatment of atopic dermatitis: a pilot study. Allergy Asthma Proc. 29, 530–537.
Shibata, T., Fujimoto, K., Nagayama, K., Yamaguchi, K., Nakamura, T., 2002. Inhibitory activity of brown algal phlorotannins against hyaluronidase. Int. J. Food Sci. Technol. 37, 703–709.
Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H., Hama, Y., 2008. Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. J. Appl. Phycol. 20, 705–711.

Shibata, T., Kawaguchi, S., Hama, Y., Inagaki, M., Yamaguchi, K., Nakamura, T., 2004. Local and chemical distribution of phlorotannins in brown algae. J. Appl. Phycol. 16, 291–296.

Shibata, T., Nagayama, K., Tanaka, R., Yamaguchi, K., Nakamura, T., 2003. Inhibitory effects of brown algal phlorotannins on secretory phospholipase A_2s, lipoxygenases and cyclooxygenases. J. Appl. Phycol. 15, 61–66.

Shim, S.Y., Quang, T.L., Lee, S.H., Kim, S.K., 2009. *Ecklonia cava* extract suppresses the high-affinity IgE receptor, FcεRI expression. Food Chem. Toxicol. 47, 555–560.

Singh, I., Bharate, S., 2006. Phloroglucinol compounds of natural origin. Nat. Prod. Rep. 23, 558–591.

Sugiura, Y., Matsuda, K., Okamoto, T., Kakinuma, M., Amano, H., 2008. Anti allergic effects of the brown alga *Eisenia arborea* on Brown Norway rats. Fisheries Sci. 74, 180–186.

Sugiura, Y., Matsuda, K., Yamada, Y., Nishikawa, M., Shioya, K., Katsuzaki, H., Imai, K., Amano, H., 2006. Isolation of a new anti-allergic phlorotannin, phlorofucofuroeckol-B, from an edible brown alga, *Eisenia arborea*. Biosci. Biotechnol. Biochem. 70, 2807–2811.

Tengamnuay, P., Pengrungruangwong, K., Pheansri, I., Likhitwitayawuid, K., 2006. Artocarpus lakoocha heartwood extract as a novel cosmetic ingredient: evaluation of the *in vitro* anti tyrosinase and *in vivo* skin whitening activities. Int. J. Cosmet. Sci. 28, 269–276.

Thiele, J.J., Podda, M., Packer, L., 1997. Tropospheric ozone: an emerging environmental stress to skin. Biol. Chem. 378, 1299–1305.

van de Laar, F.A., Lucassen, P.L., Akkermans, R.P., van de Lisdonk, E.H., Rutten, G.E., van Weel, C., 2005. α-Glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care 28, 154–163.

Welsch, C.A., Lachance, P.A., Wasserman, B.P., 1989. Dietary phenolic compounds: inhibition of Na^+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles. J. Nutr. 119, 1698–1704.

Whitaker, D., Carlson, G., 1975. Anti-Inflammation mechanism of extract from *Eisenia bicyclis* (Kjellman) setchell. J. Pharm. Sci. 64, 1258–1259.

WHO, 2010a. Factsheet: Diabetes. World Health Organization, Geneva. http://www.who.int/mediacentre/factsheets/fs312/en/index.html (accessed October 2010).

WHO, 2010b. HIV/AIDS. World Health Organization, Geneva http://www.who.int/hiv/topics/universalaccess/en/index.html (accessed November 2010).

Wijesekara, I., Yoon, N.Y., Kim, S.K., 2010. Phlorotannins from *Ecklonia cava* (Phaeophyceae): biological activities and potential health benefits. Biofactors 36, 408–414.

Xie, Z., Wang, Y., Liu, Y., Liu, Y., 2009. Ultraviolet-B exposure induces photo-oxidative damage and subsequent repair strategies in a desert cyanobacterium Microcoleus vaginatus Gom. Eur. J. Soil Biol. 45, 377–382.

Yang, H., Zeng, M., Dong, S., Liu, Z., Li, R., 2010. Anti-proliferative activity of phlorotannin extracts from brown algae *Laminaria japonica* Aresch. Chin. J. Oceanol. Limnol. 28, 122–130.

Yoon, N.Y., Chung, H.Y., Kim, H.R., Choi, J.S., 2008a. Acetyl and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. Fisheries Sci. 74, 200–207.

Yoon, N.Y., Kim, H.R., Chung, H.Y., Choi, J.S., 2008b. Anti-hyperlipidemic effect of an edible brown algae, *Ecklonia stolonifera*, and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Arch. Pharm. Res. 31, 1564–1571.

Yoon, N.Y., Lee, S.H., Li, Y., Kim, S.K., 2009. Phlorotannins from *Ishige okamurae* and their acetyl- and butyrylcholinesterase inhibitory effects. J. Funct. Foods 1, 331–335.

Yuan, Y.V., Walsh, N.A., 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food Chem. Toxicol. 44, 1144–1150.

Zhang, C., Kim, S.K., 2009. Matrix metalloproteinase inhibitors (MMPIs) from marine natural products: the current situation and future prospects. Mar. Drugs 7, 71–84.

Zhang, Y., Lee, E.T., Devereux, R.B., Yeh, J., Best, L.G., Fabsitz, R.R., Howard, B.V., 2006. Prehypertension, diabetes, and cardiovascular disease risk in a population-based sample: the Strong Heart Study. Hypertension 47, 410–413.