CHAPTER 1

INTRODUCTION

Marine algae or seaweeds such as red (Division Rhodophyta), brown (Division Phaeophyta) and green (Division Chlorophyta) algae are being used as a source of food and materials for industrial uses. The Chinese used seaweed for medicinal purposes as early as 3000 B.C. (1). Traditional medicines in Asia have used the marine algae for the treatment of cancer and to provide many health benefits (2,3). Recent research has pointed out that the consumption of marine alga could inhibit the occurrence of some inflammatory disorders, breast cancer and reduce cholesterol and hypertension (4).

1.1 Free radicals

Free radicals are defined as any atom or molecular species capable of independent existence that contain one or more unpaired electrons in its outer orbital (5). A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. They are highly reactive, unstable molecules that react rapidly with adjacent molecules via a variety of reactions including: hydrogen abstraction (capturing), electron donation and electron sharing (6). For example, the process of capturing an electron involves reacting with a donor molecule, which loses an electron and is said to have been oxidized (5). The oxidized donor molecule then has the capacity to oxidize other molecules and, thus, sets up a chain reaction (self-propagation reactions). Although free radicals play an essential role in the body, they also can react with DNA, protein or lipids in the cell membrane and cause damage (7).

1.2 Sources of free radicals

The free radicals include reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) (8,9). ROS are found intracellularly as well as extracellularly and may be produced endogenously or arise from exogenous sources, i.e. taken in from the environment (10). Endogenously, they are mainly formed as by products of electron transport chains of cell perspiration in mitochondria (11). Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted to these reactive oxygen species (12,13). Formation of ROS also occurs in different enzymatic reactions of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and other oxidizing enzymes in phagocytes, fibroblasts, smooth muscle cells and endothelial cells (14,15). Phagocyte NADPH oxidase generating superoxide radicals is the most significant source of ROS production in the vascular system; xanthine oxidase, myeloperoxidase and nitric oxide synthases are examples of other enzymes that produce reactive oxygen and nitrogen species in human body. Autoxidation reactions of molecules such as catecholamines, tetrahydrofolates, quinones, thiols or flavins (14) also produce free radicals capable of initiating oxidative events. Lipoxygenases and cyclooxygenases are involved in the synthesis of leukotrienes and prostaglandins, which have functions e.g. in mediating inflammation and regulating vascular tone and platelet aggregation; some of these enzymes are capable of oxidizing LDL (15). Transition metals iron and copper catalyse oxidative reactions in the human body. Even if the amounts of free transition metal ions in body are low, iron stored in heme, hemoglobin or myoglobin has potential to initiate oxidative reactions (16,17).

1.3 Reactive oxygen species

ROS can be classified into oxygen-centered radicals such as superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), alkoxyl radical ($RO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$) and oxygen-centered non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Other common reactive species are nitrogen species such as nitric oxide ($NO^{\bullet}$), nitric dioxide ($NO_2^{\bullet}$), and peroxynitrite ($OONO^-$) (18-20).

Excessive amounts of ROS may be harmful because they cause lipid oxidation, protein oxidation, DNA strand breaks, and modulation of gene expression (20-22). These can lead to cell injury and death, oxidative stress and numerous diseases and disorders such as cancer, stroke, myocardial infarction, diabetes mellitus, aging, gastric ulcers, inflammatory diseases, Alzheimer's and Parkinson's diseases (23-25). A balance between oxidants and antioxidants in the body is essential to avoid oxidative stress. Oxidative stress is imposed on the cell as a result of decreased levels of antioxidants. This can be caused intrinsically, for example, by DNA mutations that have altered the cellular antioxidant defense system activity, or extrinsically by a deficiency in dietary minerals (cofactors), or by toxins and other factors which deplete the antioxidant defenses. An increased level of oxidants in the cell can also result in oxidative stress.

1.4 Oxidative stress

The term oxidative stress refers to a serious imbalance between production of reactive species and antioxidant defense (26). Sies defined it as a disturbance in the prooxidant antioxidant balance in favor of the former, leading to potential damage (10). Possible causes of oxidative stress are: (26)

1. Diminished levels of antioxidants by
   - mutation of antioxidants defense enzymes: superoxide dismutase, glutathione peroxidase
   - toxins that deplete antioxidant defenses.
   - deficiencies in dietary minerals (e.g. Zn^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Se) and/or antioxidants can also cause oxidative stress.
2. Increased production of reactive oxygen species by
   - exposure of cells or organisms to elevated levels of O_2
   - toxins that are themselves reactive species or are metabolized to generate reactive species
   - excessive activation of "natural" systems producing such reactive species (e.g. inappropriate activation of phagocytic cells in chronic inflammatory diseases)

Consequences of oxidative stress can include (26)

1. Adaptation of the cell or organism by upregulation of defense systems, which may (a) completely protect against damage; (b) protect against damage to some extent but not completely; or (c) “overprotect” (e.g. the cells is then resistant to higher levels of oxidative stress imposed subsequently).

2. Cell injury: This involves damage (oxidative damage) to any or all molecular targets: lipids, DNA, protein, carbohydrate, etc.

3. Cell death: The cell may (a) recover from the oxidative damage by repairing it or replacing the damaged molecules, or (b) it may survive with persistent oxidative damage or (c) oxidative damage, especially to DNA, may trigger cell death, by apoptosis or necrosis.

Oxidative stress may result in adaptation of the cell or organism by triggering up-regulation of the immune defense system; however, this can also result in cell injury and cell death. Cellular interaction with ROS results in damage to DNA molecules, indicating that oxidative stress likely plays an important role in increasing the risk of cancer through enhanced mutagenesis, carcinogenesis, and aging (26). Oxidative stress can also elicit structural and compositional alterations to enzymes, receptors, and transport proteins that affect their functions. These defective proteins are degraded and removed from the cell (27). Some of the biological damage caused by ROS in the human body is outlined in Figure 1.

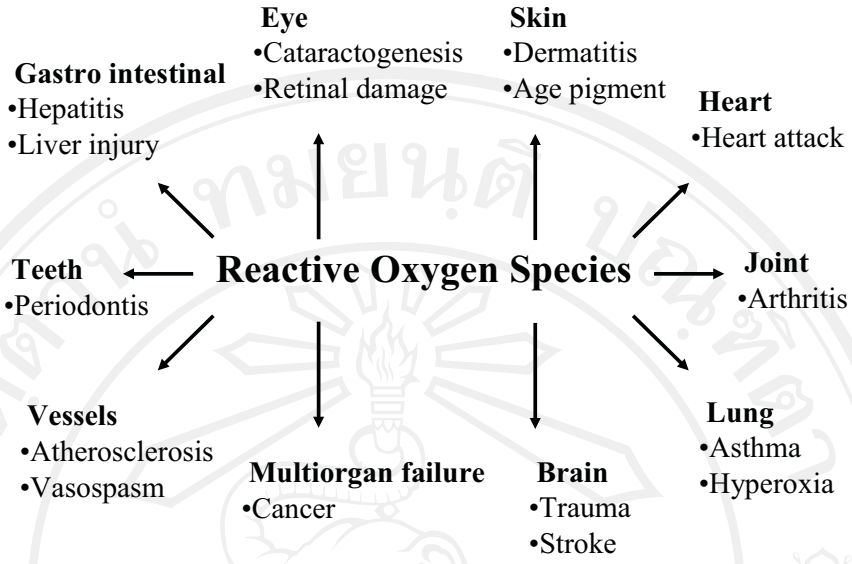

Figure 1 Biological damage caused by ROS in human body (28).

Because antioxidant defense in the human body is not completely efficient, increased free radical formation may produce a continuous level of oxidative damage (14). Oxidative stress refers to a severe disturbance in the prooxidant-antioxidant balance in favor of the prooxidant, leading to potential damage (10).

1.5 Lipid peroxidation

Lipid peroxidation, whether occurring *in vivo* or *in vitro*, has three distinct steps: initiation, propagation, and termination. The initiation step involves the reaction between free radicals ($R^{\bullet}$) and polyunsaturated fatty acids or lipid molecules (LH) in which a hydrogen atom is extracted from the fatty acid or lipid molecule to produce an alkoxyl radical ($L^{\bullet}$) (Equation 1).

$$R^{\bullet} + LH \rightarrow RH + L^{\bullet} \qquad (1)$$

The alkoxyl radical will be further oxidized during the propagation step to form a peroxyl radical ($LOO^{\bullet}$), which further extracts a hydrogen atom from another

fatty acid or lipid molecule. These products can easily interact with nearby lipid molecules and produce secondary lipid radicals (Equation 2-4).

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (2)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (3)$$

$$LOO^{\bullet} \rightarrow LO^{\bullet} + {}^{\bullet}OH \quad (4)$$

The free radical production in the propagation step will be continued until the termination step takes place. The final step involves the combination of two free radicals, which results in a non-radical product (29,30).

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{Non-radical products} \quad (5)$$

$$L^{\bullet} + LOO^{\bullet} \rightarrow \text{Non-radical products} \quad (6)$$

$$L^{\bullet} + L^{\bullet} \rightarrow \text{Non-radical products} \quad (7)$$

Of all biological products, lipid oxidation products have one of the longest half-lives (10^3 to 10^6 times) and, therefore, are predisposed to attacking critically important cellular components and increasing the potential of cell damage that causes different disorders, such as atherosclerosis and cardiovascular disease. These situations may be potentially prevented by consuming dietary antioxidants, including anthocyanins, which work by counteracting the imbalance of oxidative agents present in the body (26).

Lipid peroxidation represents one form of tissue damage associated with disease states and drug-induced toxicity that proceeds by free radical-initiated chain reactions.

1.6 Antioxidants

Antioxidants are substances which counteract free radicals and prevent the damage caused by them. These can greatly reduce the adverse damage due to oxidants by crumbling them before they react with biologic targets, preventing chain reactions or preventing the activation of oxygen to highly reactive products (31).

Antioxidants can act at different levels in the oxidative sequence. As far as lipid peroxidation is concerned, they could act by:

1. Decreasing localized O_2 concentrations (e.g. sealing of food stuffs under nitrogen)
2. Preventing first-chain initiation by scavenging initiating radicals such as $^{\bullet}OH$
3. Binding metal ions in form that will not generate such initiating species as $^{\bullet}OH$, ferryl, or $Fe^{3+}/Fe^{2+}/O_2$ and/or will not decompose lipid peroxides to peroxyl or alkoxyl radicals
4. Decomposing peroxide by converting them to non-radical products, such as alcohol
5. Chain breaking, i.e. scavenging intermediate radicals such as peroxyl and alkoxyl radicals to prevent continued hydrogen abstraction. Chain-breaking is often phenols and amines

Antioxidants acting by mechanisms 1, 2 and 3 can be called preventive antioxidants. Those acting by mechanism 3 are not usually consumed of the course of the reactions. Antioxidants of the fourth type are also preventive antioxidants, but they may or may not be consumed during the reaction, depending on their chemical behavior (e.g. glutathione peroxidase acts by this mechanism and being an enzyme, is a catalyst and is not consumed). Chain breaking antioxidants, acting by combining with the intermediate radicals, will be consumed, as will antioxidants of type 2 as shown above. It should be stressed that many antioxidants have multiple mechanisms of action (32,33).

1.7 Biological antioxidants

Biological antioxidant sources can be classified into two major groups (Figure 2). Enzymatic antioxidants, which are produced endogenously to protect the body from the adverse effects of free radicals, such as superoxide dismutase (in mitochondria and the cellular cytosol), catalase (in peroxisomes) and glutathione peroxidase (in the cell membrane). Non-enzymatic or dietary antioxidants, which are external antioxidant sources that combat oxidation when internal defense systems fail, due to a decrease in cellular antioxidants or an increase in the production of free

radicals. Antioxidant enzyme cofactors (e.g. coenzyme Q_{10}), transition metal chelators (e.g. EDTA), and radical scavengers (e.g. vitamins and polyphenols) are different examples of non-enzymatic antioxidants (34, 35).

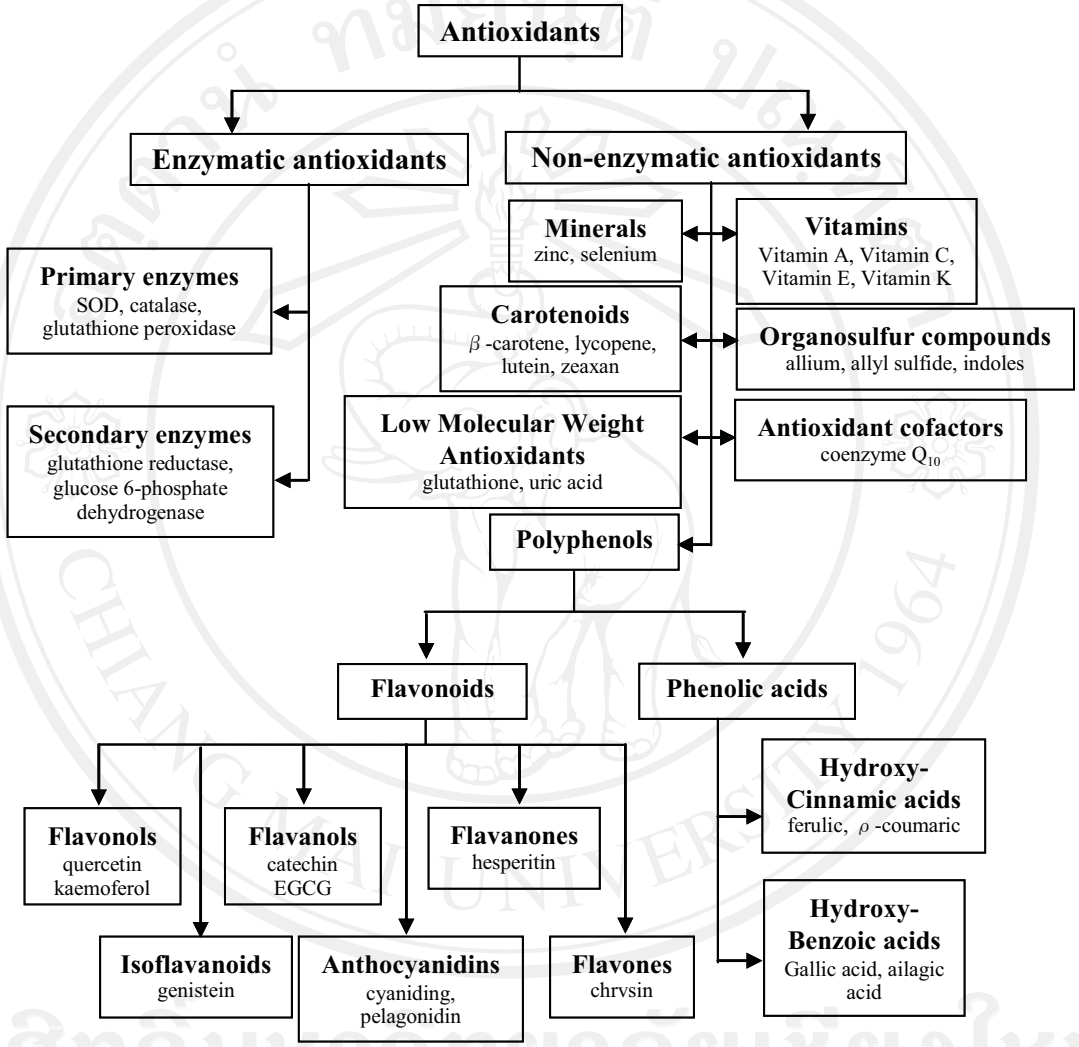

Figure 2 Classification of antioxidants (36)

1.8 Antioxidant activity assays

1.8.1 $ABTS^{\bullet+}$ radical scavenging activity assay

The ABTS assay was first reported by Miller *et al*. (37). This spectrophotometric method is also known as the trolox equivalent antioxidant capacity (TEAC). ABTS (2,2'-azinobis-[3-ethylbenzothiazoline-6-sulphonic acid]) is a peroxidase substrate, which when oxidized by peroxyl radicals or other oxidants in the presence of H_2O_2 generates a metastable radical cation ($ABTS^{\bullet+}$) which is

intensely colored and can be monitored spectometrically in the range of 600-750 nm. The antioxidant capacity is measured as the ability of test compounds to decrease the color reacting directly with $ABTS^{\bullet+}$ radical and expressed relative to Trolox (a derivative of vitamin E) (38).

In further modified methods that utilize $ABTS^{\bullet+}$ radical in common, ABTS was generated by peroxidase, myoglobin or chemical reactions (manganese dioxide, potassium persulfate). Generally, chemical reactions require a long time (e.g. up to 16 h for potassium persulfate generation), whereas enzymatic reaction is faster and the reaction conditions are milder (39).

1.8.2 $DPPH^{\bullet}$ radical scavenging activity assay

The DPPH radical is a long-lived organic nitrogen radical and has a deep purple color. It is commercially available and does not have to be generated before assay. In this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by electron spin resonance or by monitoring the absorbance decrease at 515-528 nm until the absorbance remains stable in organic media. This widely used method was first reported by Brand-Williams et al. (40).

1.8.3 Superoxide anion radical scavenging activity assay

Superoxide anion radical ($O_2^{\bullet-}$) formed *in vivo* is largely converted by superoxide dismutase (SOD)-catalysed or nonenzymic dismutation into H_2O_2 (41). It is easily produced by radiolysis of water in the presence of O_2 and formate, and these techniques allow examination of the spectrum of products formed when $O_2^{\bullet-}$ reacts with putative antioxidants (42).

1.8.4 Hydroxyl radical ($^{\bullet}OH$) scavenging activity assay

In the deoxyribose oxidative degradation assay, $^{\bullet}OH$ radical is produced in aqueous reaction solution containing $FeCl_3$ and H_2O_2 by Fenton reaction. Nitrilotriacetic acid is included in the reaction mixture for their metal chelating properties. To assess the degree of deoxyribose degradation, the oxidative degradation is condensated with thiobarbituric acid at low pH, and quantified by spectrophotometrical measurements at 532 nm (43).

1.8.5 Peroxyl radical scavenging assay

Formation of peroxyl radicals ($RO_2^{\bullet}$) is a key step in lipid peroxidation (33), but they can also be formed from DNA and proteins (44,45) and when thiyl ($RS^{\bullet}$) radicals combine with oxygen (45-47). $RO_2^{\bullet}$ radical scavengers may be water soluble (e.g. dealing with radicals from DNA, thiols, proteins) or lipid soluble (e.g. the chain-breaking antioxidant inhibitors of lipid peroxidation).

1.8.6 Anti-lipid peroxidation assay

To test lipid peroxidation activity directly, one can simply examine the ability of an antioxidant to inhibit peroxidation of lipoproteins, tissue homogenates, fatty acid/ester emulsions, liposomes or membranes (e.g. erythrocytes, liposomes, microsomes).

β-carotene bleaching model

Carotenoids bleach via autoxidation, oxidation induced by light or heat, or oxidation induced by peroxyl radicals, and this decolorization can be diminished or prevented by classical antioxidants that donate hydrogen atoms to quench radicals. Although β-carotene is often used as the target, its decolorization at 470 nm can occur by multiple pathways, so interpretation of results can be complicated (48).

Thiobarbituric Acid Reacting Substances (TBARS) assay

The TBARS test is widely used. Lipid peroxidation is often started by adding metal ions such as Fe^{2+}, $FeCl_3$ plus ascorbate. In these cases, an antioxidant effect could occur not only by peroxyl radical scavenging but also by metal ion chelation (49).

1.8.7 Metal chelating assay

In the assay system, ferrozine can quantitatively complex with Fe^{2+} to red colour, and in the presence of chelating agents, the complex formation is inhibited and the red colour of the complex fades. Measuring of the colour reduction, therefore, it is possible to estimate of the chelating activity of the co-existing chelator (50).

1.8.8 Reducing power assay

This spectrophotometrically assay is also known as the Ferric Reducing Antioxidant Power (FRAP). There are no free radicals introduced into system. The FRAP assay uses an oxidation/reduction reaction to measure the ability of a sample to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The mechanism of this method is

different from free radical-scavenging assay. This method involves neither a pro-oxidant nor an oxidizable substrate. The antioxidant activity of investigated samples determined by FRAP assay base on their ability to reduce oxidizing species or oxidants (50).

1.8.9 Hemoglobin-induced linoleic acid peroxidation inhibition

The inhibition of hemoglobin-induced linoleic acid peroxidation was determined by a spectrophotometric assay. The autooxidation was started by adding hemoglobin to linoleic acid. The lipid peroxidation was stopped by adding HCl/ethanol. This method could evaluate the results with only 1 h of oxidation time. General antioxidant assays with linoleic acid need more autooxidation for 5-6 days (51).

1.9 Antioxidant properties of marine algae

Various marine algae with antioxidant properties have been reported, as summarized in Table 1.

Table 1 List of marine algae which have been reported to have antioxidant properties

Marine algae	Antioxidant activity assays	References
Division Phaeophyta		
Chorda filum	DPPH$^{\bullet}$ and $^{\bullet}$OH scavenging activity	(52)
Colpomenia sinuosa	DPPH$^{\bullet}$, $ABTS^{+\bullet}$, H_2O_2 and $^{\bullet}NO$ scavenging activity, total antioxidant capacity	(53)
Desmarestia viridis	DPPH$^{\bullet}$, $^{\bullet}$OH scavenging activity	(52)
Ecklonia cava	DPPH$^{\bullet}$, $^{\bullet}OH$, H_2O_2, $^{\bullet}O_2$ and $^{\bullet}NO$ scavenging activity, metal chelating activity, reducing power, alkyl radical scavenging; comet, lipid peroxidation inhibition	(54, 55)
Fucus vesiculosus	$O_2^{-\bullet}$ and $^{\bullet}$OH scavenging activity	(54)
Hijikia sp.	β-carotene bleaching, DPPH$^{\bullet}$ scavenging	(55)

	activity	
Hizikia fusiformis	DPPH$^{\bullet}$, $O_2^{-\bullet}$ and $^{\bullet}$OH scavenging activity, lipid peroxidation inhibition	(56)
Lobophora variegata	DPPH$^{\bullet}$ scavenging activity	(57)
Padina antillarum	DPPH$^{\bullet}$ scavenging activity, reducing power, metal chelating activity, beta carotene bleaching	(58)
Padina gymnospora	$O_2^{-\bullet}$ and $^{\bullet}$OH scavenging activity	(54)
Padina minor	DPPH$^{\bullet}$ and $ABTS^{+\bullet}$ scavenging activity, lipid peroxidation	(59)
Papenfussiella kuromo	hemoglobin-induced linoleic acid peroxidation inhibition, reducing power, metal chelating activity, DPPH$^{\bullet}$ and $O_2^{-\bullet}$ scavenging activity	(51)
Sargassum kjellmanianum	DPPH$^{\bullet}$ and $^{\bullet}$OH scavenging activity	(52)
Sargassum siliquastrum	RBC hemolysis and lipid peroxidation inhibition	(60)
Sargassum thunbergii	DPPH$^{\bullet}$ and $^{\bullet}$OH scavenging activity, alkyl radical scavenging	(61)
Scytosiphon lomentaria	hemoglobin-induced linoleic acid peroxidation, reducing power, metal chelating activity, DPPH$^{\bullet}$ and $O_2^{-\bullet}$ scavenging activity	(51)
Undaria sp.	β-carotene bleaching, DPPH$^{\bullet}$ scavenging activity	(55)
Division Chlorophyta		
Avrainvillea longicaulis	DPPH$^{\bullet}$ scavenging activity	(57)
Caulerpa racemosa	DPPH$^{\bullet}$ scavenging activity, reducing power, metal chelating activity, β-carotene bleaching	(58)
Caulerpa sertularoides	metal chelating activity	(62)
Laminaria japonica	$O_2^{-\bullet}$ and $^{\bullet}$OH scavenging activity	
(	6	3)
Laminaria sp.	β-carotene bleaching, DPPH$^{\bullet}$ scavenging activity	(55)
Ulva latutus	DPPH$^{\bullet}$ scavenging activity, β-carotene bleaching	(64)

Ulva pertusa (65)	$O_2^{-\bullet}$ and $^{\bullet}OH$ scavenging activity, reducing power, metal chelating activity	
Division Rhodophyta		
Chondria baileyana	$DPPH^{\bullet}$ scavenging activity	(57)
Gelidium amansii	$DPPH^{\bullet}$ and $^{\bullet}OH$ scavenging activity	(52)
Gloiosiphonia capillaris	$DPPH^{\bullet}$ and $^{\bullet}OH$ scavenging activity	(52)
Grateloupia filicina (66)	$DPPH^{\bullet}$, H_2O_2 and $O_2^{-\bullet}$ scavenging activity, lipid peroxidation inhibition	
Kappaphycus alvarezii	$DPPH^{\bullet}$ scavenging activity, metal chelating activity, reducing power, β-carotene bleaching	(58,67)
Polysiphonia urceolata	$DPPH^{\bullet}$ scavenging activity, β-carotene bleaching	(52,68)
Porphyra haitanensis	lipid peroxidation inhibition, superoxide dismutase activity and glutathione peroxidase	(69)
Porphyra sp.	β-carotene bleaching, $DPPH^{\bullet}$ scavenging activity, hemoglobin-induced linoleic acid peroxidation, reducing power, metal chelating activity, $O_2^{-\bullet}$ scavenging activity	(55, 51)
Rhodomela teres	$DPPH^{\bullet}$ and $^{\bullet}OH$ scavenging activity	(52)
Symphyocladia latiuscula	$DPPH^{\bullet}$ scavenging activity	(70)

1.10 *Gracilaria fisheri* (Xia & Abbott), Zhang & Xia, a red marine alga

Gracilaria fisheri (Xia & Abbott), Zhang & Xia (known by the common name of phomnang) is a red marine alga from Division Rhodophyta, family Gracilariaceae, and is found abundance in the southern part of Thailand (Figure 3). It is a thallus bushy, 13-30 (up to 45) cm tall, with many branches coming from a short stipe or from current axis; branching alternate, three to four orders; branches cylindrical, main branches 0.6-2.3 mm in diameter, constricted at bases and tapering toward apices. *G. fisheri* was found growing on living and empty shells (*Cerithium sp.*) and on broken rocks, gravel, polyethylene bags, and nets of fish cages in sandy-muddy areas of turbid water (71).

Figure 3 *Gracilaria fisheri* Xia & Abbott (Family Gracilariaceae, Division Rhodophyta)

1.11 Biological activities of *Gracilaria fisheri* and other *Gracilaria* species

Quite a few studies on biological activities of *G. fisheri* have been reported. An aqueous extract of *G. fisheri* showed hypotensive activity when administered in normotensive anesthetized rats (72). Previously, the aqueous extract of *G. fisheri* was found to have the $DPPH^{\bullet}$ scavenging activity thus suggesting it possesses an antioxidant activity (73).

Antioxidant properties of various species of *Gracilaria* have been reported. The methanolic extract of *G. changii* showed $DPPH^{\bullet}$ scavenging activity and the presence of phenolic contents (74). Crude methanol extract of *G. edulis* was found to have a reducing power and contain phenolic content (75). Ascorbate, superoxide dismutase, glutathione and catalase which are known to possess antioxidant capacity were found in *G. vermiculophylla* (76).

In addition to antioxidant activity, other biological activities of various species of *Gracilaria sp.* have been found.

Prostaglandin E_2 (PGE_2) from *G. lichenoides* exerted an antihypertensive effect when administered intravenously to hypertensive rats (77).

G. verrucosa were suggested to have anti-inflammatory activity according to the results from the study of which the effects of extracts on inflammatory markers [tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS), and cyclooxygenases (COXII)] in macrophage cell line RAW264.7 were evaluated. Additionally, enone fatty acids from *G. verrucosa* showed potential anti-inflammatory activity, causing inhibitory effects on the production of pro-inflammatory mediators [nitic oxide (NO), IL-6, and TNF-α] in lipopolysaccharide (LPS)-activated RAW264.7 murine macrophage cells (78).

The aqueous extract of *G. corticata* showed hepatoprotective potential against aflatoxin B1 (AFB1) induced hepatotoxicity (79). Galactan sulfate from *G. corticata* exhibited antiviral activity against herpes simplex virus types 1 and 2 (80).

1.12 Antihypertensive effect of marine algae

Hypertension is the most common cardiovascular disease. Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principle cause of stroke, leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death, and is a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the aorta (81).

Antihypertensive activities of marine algae have been reported. In randomized, case-controlled study, administration of dried powder wakame (*Undaria pinnatifida*) in hypertensive patients at a dose of 5 g/day for 8 weeks caused significant decreases of both systolic and diastolic blood pressure, and hypercholesterolemia (82). Furthermore, peptides with angiotensin converting enzyme inhibitory activity which exhibited antihypertensive effect when tested in spontaneously hypertensive rats have been found in wakame (83,84). Additionally, other marine algae: *Chlorella vulgaris* and *Spirulina platensis* were found to show antihypertensive effect when their peptidic fractions were tested in spontaneously hypertensive rats (85).

1.13 Purposes of the study

The present work will be carried out to evaluate *G. fisheri* for antioxidant activity of which various testing models will be employed. Additionally, *G. fisheri* will be investigated for antihypertensive effect and effects on the heart and blood vessel.

The purposes of the present study are:

1. To investigate antioxidative activity of *G. fisheri* which including
    1.1 radical scavenging activity ($ABTS^{+\bullet}$, $O_2^{-\bullet}$, $^{\bullet}OH$)
    1.2 anti-lipid peroxidation activity
    1.3 metal chelating activity
    1.4 reducing power
2. To determine the phenolic contents of *G. fisheri*
3. To evaluate *G. fisheri* for antihypertensive effect
4. To study the effect of *G. fisheri* on isolated heart
5. To study the effect of *G. fisheri* on isolated blood vessel