SOCIETY FOR SCIENCE AND EDUCATION UNITED KINGDOM

JBEMi JOURNAL OF BIOMEDICAL ENGINEERING AND MEDICAL IMAGING

ISSN: 2055-1266

VOLUME 6 ISSUE 5

Effect of Different Processing Conditions on Antioxidant Activity of *Gracilaria Edulis*(Rhodophyseae) in Sri Lanka

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ABSTRACT

Gracilaria edulis is red marine algae currently cultivated in Sri Lanka but relatively low than other country such as China and Japan. This species are used to produce drugs and healthy delicious low calorie foods because of they are primary source of secondary metabolite and to be used as natural antioxidants and antimicrobials. The Purpose of this research investigates effect of different processing conditions on antioxidant activity of *G.edulis* (Rhodophyseae) by hydrogen peroxide scavenging activity assay. Absorbance at 230 nm, hydrogen peroxide scavenging assays were done. Different processed *G.edulis* such as fresh, steamed, boiled, dried and microwaved were extracted with methanol. Their antioxidant properties were compared to the L-ascorbic acid which used as positive control. The methanolic extracts of this red algae was prepared keeping methanol as a standard. Methanol was used as control. In minitab-15 statistical software package, Paired T-Test was clearly shown the absorbance, inhibition or hydrogen peroxide scavenging activity depend on the processing conditions of the *G.edulis* methanol extract. This statistical test did tell about the difference between the different processing conditions of the methanol extract of the *G.edulis* ($p<0.05$) and antioxidant activity and these were not independent factors such are depend on each other. Hence antioxidant activity or H_2O_2 scavenging activity was highly in boiled *G.edulis*, followed by fresh, microwaved, dried and steamed respectively. L-ascorbic acid was used as Positive control and its antioxidant activity was higher than dried and steamed but relatively lowers than microwaved, fresh and boiled *G.edulis*. Finally Boiled *G.edulis* sample was recorded prominent antioxidant activity due to leaching of less antioxidant compounds. In future this study is used to functional product development incorporation of dried followed by boiled G*. edulis* into bakery Products.

***Key words*:** Antioxidant, scavenging, absorbance, processed, Inhibition.

1 Introduction

Seaweeds are commonly called as marine algae which are broadly categorized into 45000 species in worldwide (Amin, 2002). Bioactive compounds of seaweeds are having various biological activities (Bouhlar, et al., 2011,).which are derived the secondary metabolites to screen antimicrobial and antioxidant activities (Yuan *et al.*, 2005; Bansemir et al., 2006; Chew et al., 2008). More than 600 secondary metabolites were been isolated from Seaweeds as extensive profile source and provide wide

DOI: 10.14738/jbemi.65.8007
Publication Date: 21st Oct 2019
URL: http://dx.doi.org/10.14738/jbemi. 65.8007

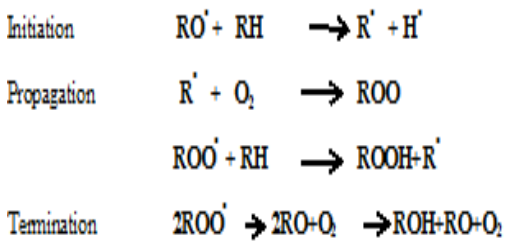
range of biological activites (Itoc & Aorik.,1989; Pisani, et al., 1990). Among the red seaweeds particularly *G.edulis* consist Primary and secondary metabolities (Layse, 2011).

China and Japan people were consumed some type of seaweeds since 300 B.C. Among the countries in the world, edible seaweeds are cultivated highly by these two countries (Arasaki, 1984; Chengkui, 1984). Most of the consumable seaweeds especially under Rodophyceae, *G.edulis* was contained high level of dietary fiber, indigestible carbohydrates, vitamins, minerals and essential amino acids (Layase, et al., 2011). Such type of seaweeds contain bio active compounds that are used in food industries and pharmaceutical industries and used as diet in china and Japan compare to Europe and North America for cure the prostrate and breast cancer (skiboda, 2004). One or more unpaired electrons means single electrons contain in free radical molecule outer orbital. Such types of free radical are in reactive oxygen and nitrogen species. These were generated by oxidative stress brought about by free radical formulation and imbalance of body antioxidant defense system (Meenakshi, et al., 2009). Oxidative stress cause ischemic injury, cancer aging inflammation, and neurodegenerative diseases (Parkinson's and Alzheimers's). Reactive oxygen species such as nitric oxide radical, superoxide radical, peroxyl radical and hydroxyl radical form extensive oxidative damage are associated with those diseases. Such reactive oxygen species attach with RNA and DNA, Lipids and enzymes leading to cell or tissue damage and carcinogenesis and which are associated with chronic diseases such as cerebrovascular and cardiac diseases (Reaven and Witzum, 1996; Aruoma, 1999).

Seaweeds produce strong oxidizing agents and free radicals in high oxygen concentration and light environment. This is happen in photodynamic damage during metabolism period. Bioactive compounds of consumable seaweeds have protective mechanisms against the free radicals (Matsukawa, et al., 1997). Antioxidants neutralize free radicals (Rinelhof, et al., 2000). Antioxidants are found as micronutrients which are scavenged or neutralized free radicals for protect from free radical induced diseases. (Chanda, 2010). Bioactive substances extract from *G.edulis* can have ability to destroy free radicals. This protection was measured by presence of antioxidants. This effect could be evolved by metal ion chelators, quenchers, free radical scavengers, hydrogen donating compounds. (Amin and Hong, 2002). In the family Rhodophyta, especially *G.edulis* are the major Sri Lankan agarophytes and to be considered as very important species for biotechnologies industries. This species is used to prepare agar and agarose. Antimicrobial activities, antiviral, antifungal and antioxidant activities have been screened in green, brown and red algae (De souza, et al., 2007; smith, 2004., Madhusudan, et al., 2011). The objective of this present research is used to study the effect of different processing conditions on antioxidant activity of *G.edulis* species.

Oxidative deterioration is taken place in lipid form of phospholipid and tryglyceride and then generated food quality deterioration, off flavours rancidity and other reactions. Light, heat, metalion/metalloprotein and ionizing radiation are initiated autooxidation. Lipoxygenaseis process is taken by three steps such as initiation, propogation and termination. Which three steps are occurred in autooxidation process (Shihidi & Naczk, 2004).

General scheme for autooxidation of polyunsaturated lipids:



Human physiopathologies involve with reactive oxygen species (Shahidi and Naczk, 2004). Oxidative stress is a reason to form many multifactorial diseases such as inflammatory disorders (Bodamyali, et al., 2000) especially cancers (Kawanishi, et al., 2002) cardiovascular diseases (Sachidanandame, et al., 2005). This oxidative stress caused by productions of oxidants including antioxidant systems and ROS. ROS is causing diseases such as inflammation, cancer, cardiovascular disease, artherosclerosis, arthritis, aging, diabetes and parkinsonism. In seaweeds, Phenolic phytochemicals content inhibit autoxidation of unsaturated lipids and formation of oxidized low-density lipoprotein (LDL) for protecting the body from cardiovascular diseases (Amic, et al., 2003). Therefore high level of Phytochemical content of any food may protect the human body (Chandini, et al., 2008).Oxidative alteration of bioactive molecules involve with mechanism of ROS. Such bioactive molecules are carbohydrates, nucleic acids, proteins and lipids along with the inflammatory response and modulation of gene expression. (Laguerre, et al., 2007). Nitric oxide radical ($NO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), Superoxide radical ($O^{\cdot}2^{-}$), and peroxyl radical ($ROO^{\cdot}$) are ROS. These molecules were attacked molecules such as enzymes, proteins, DNA, RNA and lipids, leading to cell damage associated with carcinogenesis aging, and atherosclerosis (Fisch, et al., 2003; Shon, et al., 2003; Nakamura, et al., 2003; Valentao, et al.,2002). Normally antioxidants found compounds are protecting the body against ROS. During the photosynthesis process in seaweed molecular oxygen evolved in the presence of solar light. This molecular oxygen reaches highest concentration level during that process. In the presence of heat or UV radiation of sunlight, oxygen activate into toxic ROS. Marine algae antioxidants have special defense mechanisms against ROS for reduce their concentration. (Lu and Foo, 1995). Antioxidants have different mechanisms for scavenging free radicals, binding metal ions, decomposing hydroperoxides and peroxides among others (Moure, et al., 2001). Free radical inactive by natural good antioxidants highly.

Table:1* Different mechanism of antioxidant activity

Antioxidant class	Examples of antioxidants	Mechanism of antioxidant activity
Proper antioxidant	Phenolic compounds, tocopherols, Flavanoids	Inactivating lipid free radicals
Hydroperoxide stabilizers	Phenolic compounds	Preventing decomposition of hydroperoxides into free radicals
Substance reducing hydroperoxides	Protein, aminoacids	Reducing hydroperoxides in a non-radical way.
Singlet oxygen quenchers	carotenoids	Transforming singlet oxygen into triplet oxygen
Synergists	Citric acid, ascorbic acid	Promoting activity of proper antioxidants
Metal chelation	Flavanoids, Phosphoric	Promoting activity of proper antioxidants

Many researchers were stated as seaweeds are rich sources of natural antioxidant compounds (Lim, et al., 2002; Duan, et al., 2006; Kuda, et al., 2007; Cox, et al., 2010). Plant-derived structurally related antioxidants such as Chlorophylls, carotenoids, tocopherol derivatives such as vitamin E and related isoprenoids were also found in some marine organisms in naturally (Takamatsu, et al., 2003). Antioxidant activity may evolve by niacin, flavonoids, ascorbic acid and pigments such as chlorophylls, carotenoids, and vitamins and vitamin precursors including ,thiamine, β-carotene α-tocopherol and phenolics such as hydroquinones, polyphenolics, and phospholipids particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances in seaweeds which are suppressed oxidation process directly or indirectly (Shahidi, 2008). Micronutrients found in red green and brown algae and act as natural antioxidants (Chanda, 2010). Phenolic compounds of seaweeds were available as benzene ring substituted by at least one hydroxyl (Manach, et al., 2004; Duan, et al., 2006). These phenolic compounds of antioxidant activities were worked as chelating metal ions and improving the antioxidant endogenous system by preventing radical formation (Al-Azzawie and Mohamed-Saiel, 2006). flavanones, flavones, flavononols, flavonols, flavan-3-ols,tocopherols, chalcones, tannins, lignins and phenolic acids are commonly polyphenols found in seaweeds. Which are source of antioxidant activities to reduce the use of BHT and BHA and natural antimicrobial (Shukla, et al., 1997).

Free radicals are caused oxidative damage or oxidative stress. These free radicals such as reactive oxygen and nitrogen species contain one or more unpaired electrons in outer orbitals and produce Parkinson disease, aging, cancer, ischemic injury, alzheimers and inflammation. Free radicals and Oxygen species oxidize protein, nucleic acid, DNA and lipid (Vadlapadi, et al., 2010). These free radicals are reduced by natural antioxidants (Akoh and Min, 1997). Phenolic acid, Carotenoids, Phytoestrogens, phyruvate, vitamin E and Vitamin C are antioxidants to neutralize free radicals from different sources (Meenakshi, et al., 2009). Naturally available antioxidants protect human from oxidative damage and increase the shelf life of foods (Chandini, et al., 2008).Tannins of natural phenolic metabolite is commonly found in marine and terrestrial plants and categorized into hydrolysable and condensed compounds (Haslam, 1989; Waterman and Mole, 1994). Phlorotannins is under Tannin compounds group. Phlorotannins derived from marine plant is having unique eight interconnected rings molecular structure and possess antioxidant activity. In marine algae phlorotannins synthesize in the acetate-malonate pathway by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units. (Ragan and Glombitza, 1986; Waterman and Mole, 1994; Arnold and Targett, 1998). Naturally available antioxidants in *G.edulis* such as flavonoid, phenolic acid and poly phenol retard or inhibit oxidative stress that leads to degenerative similar diseases (Miller, H.E. & Rinelhof, et al., 2000).

Decomposition of hydroperoxides into free radicals prevent by Flavonoids and Phenolic compounds. Phenolic compounds were proper antioxidants. Such Carotenoids molecules reduce hydroperoxides by a non-radical way. Natural antioxidants reduce toxic causing Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) (Duan, et al., 2006). Phenolic compounds, flavonoids, alkaloid, saponins and carotenoids are natural antioxidants widely distributed in the *G.edulis*. Among these bioactive substances especially Phenolic components and flavonoids contain structural requirements for free radical scavengers and natural food antioxidants (Bandoniene and Murkovic, 2002). Which act as a protective mechanism against oxidative damage of living cell (Vimala, et al., 1999) and also increases the shelf-life of foods (Schwarz, et al., 2001).

Environmental stress and microbes affects are protected by protective mechanisms of phenolic compounds (Harbourne, 1994; Herrmann, 1989; Wallace and Fry, 1994). Flavonoid is one of largest group of phenolic compounds. This Flavonoid has free radical scavenging activities (Kahkonen, et al., 1999). Because of radicals planar conformation offers extended electronic delocalization between adjacent rings, Flavonols were exposed antioxidant activity while study the quantum mechanical (Russo, et al., 2000). Flavanol was present in *G.edulis*. (Umakanthan, et al., 2016).

Antioxidant activity or inhibition activity or radical scavenging activity of antioxidants were measured by several analytical methods such as oxidation rate, % of the reagents used and inhibition against free radicals like hydroxyl radical(OH), 1,1-diphenyl-2-picrylhydrazyl(DPPH), hydrogen donating compounds metal chelators (Amin and Hong, 2002). While consider this method for measure antioxidant activity, primary radicals are reduced to non-radical chemical compounds into oxidize antioxidant radicals by donating hydrogen radicals (Jadhav, et al., 1995;Yamaguchi, et a., 1998; Hwang. Pai. Aa, 2010; devi, et al., 2008).

Antioxidant activities or scavenging activities depend on active group presence in sample. These antioxidant activities vary with presence of active group, kind and their position. In the presence of free radicals, antioxidants can be shown two types of mechanisms.

1. $R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$

2. $R^{\bullet} + ArOH \rightarrow R + ArOH^{+}$

In the first mechanism, antioxidant activity measure by OH bond dissociation energy. Antioxidant activity of ArOH loses its hydrogen atom and become a radical. This is reaction is more suitable to explain DPPH scavenging activity. OH bond dissociation energy inversely proportional to DPPH scavenging activity and antioxidant activity (Wright, et al., 2001). Lower bond energies means weaker OH bond will be inactivated free radical easily due to weaker OH bond dissociation energy lose its hydrogen atom easily. This losing H atom reacts with free radical for free radical inactivation. In the second mechanism, antioxidant activity explains by H_2O_2 scavenging activity or H_2O_2 assay. Where ArOH is antioxidant and that gives an electron to form radical cation. Electron donor ability is proportional to H_2O_2 scavenging or antioxidant activity (Wright, et al., 2001).

Over last two decades natural antioxidant molecules has been interested for research studies. Exogenic antioxidants derived from foods are very significantly to counteracting oxidative stress. Marine plants are the major source of these antioxidants in the form of phenolic compounds such as flavonoid, carotenoid, alkaloid and saponins (Laguerre, et al., 2007). In recently among the most of, Algae, Tea, herbs, terrestrial pants, marine plants and seeds are researched to identify as natural antioxidants. But vast research knowledge has not been gained into the effect of different processing conditions on antioxidants activity resultant from *G.edulis*. Previous my research was revealed the antioxidant activity of *G.edulis* but not clearly state their effect of different processing conditions on antioxidant activity of *G.edulis*.

Seaweeds based foods have been eaten raw or after processing treatment by human in Asian countries since from ancient times. This processing techniques were been varied in different parts of world such as

steaming, boiling, drying and microwaving where number of changes were been happen in its physical characteristics and chemical composition (Zhang and Hamauzu, 2004). While processing techniques take place in vegetable prior to consumption, phytochemicals and its concentrations and detrimental factors were been considered as prime factor to alter health related quality. In wet thermal treatment, denaturation of enzymes can catalyze breakdown of phytochemicals and nutrients. The heat processing technique causes the reduction of phtochemical content due to thermal destruction or leaching (Rungapamestry, et al., 2007).Nowadays food industry and consumers use various preservation techniques and methods. Food processors, food safety regulators and regulatory agencies are continuously disturbed with increase of illness caused by some pathogenic and spoilage microorganisms in foods. Hence currently Consumers are demanding minimally processed natural, fresh and safety foods that contain natural antioxidant and synthetic preservatives to protect the body from those multifactorial diseases. This is the reason behind food industries are more concerned in the research for discover alternative natural antioxidant (Shan, et al., 2007). Research results on the effects of processing on the antioxidant compounds in seaweeds have been inconclusive. While doing some research in vegetables their results revealed no change or an increase in antioxidant activity (Gahler, et al., 2003; Turkmen, et al., 2005) and some another research was shown deterioration activity after thermal treatment (Ismail, et al., 2004; Zhang and Hamazu, 2004).The phytochemicals in seaweeds are important for human health. Natural antioxidant compound of red algae especially *G.edulis* was been studied. Therefor seaweed processing technique thermally prior to consumption which matter is needed to investigate how the processing techniques affect the antioxidant activity of *G.edulis*. Bioactive substances or Phytochemicals are richly available in seaweed foods. That is responsible for antimicrobial and antioxidant activity. Previously Quantities of the biochemical compounds in seaweeds have been studied by many researchers (Duan et al., 2006; Chandini et al., 2008; Cox et al., 2010). Most of the science researchers were screened antioxidant activity of seaweed varieties (Gonzalez del Val, et al., 2001; Ganesan, et al., 2008; Plaza, et al., 2009). *G.edulis* is commonly found in Sri Lanka especially edible seaweed widespread in Kalpitiya, Puttalam. However there is no further information about effect of different processing conditions on antioxidant activity of seaweed extracts from Sri Lanka especially widespread of red seaweed *G.edulis* in Kalpitiya, Puttalam. This research was determine most favorable processing conditions could be used to exhibit high antioxidant compounds for making most potent functional food. The main purpose of this research chapter was used to study the effect of different processing conditions on the antioxidant activity of *G.edulis* by hydrogen peroxide scavenging activity.

2 Materials and Methodology

2.1 Sample collection:

G.edulis samples from Kalpitiya, Puttalam were collected and transported to the lab in 0℃ by keeping in an insulated box for assess the different processing conditions on antioxidant activities of *G.edulis*. Those particular sample specimens were identified at Department of Zoology, Eastern University, Sri Lanka.

2.2 Preparation of methanol residual extracts of *G.edulis*

The *G.edulis* samples were rinsed thoroughly in fresh water and then distilled water used to remove dirt mud, particles and other epiphytes. *G.edulis* samples were prepared as fresh, dried for 12 hours, Boiled at 100℃, steamed and microwaved 900W in 12-15minutes to assess the different processing conditions

on antioxidant activities of *G.edulis.* After that each samples were blended. Then that 10grams of blended sample was mixed with 100ml of methanol solvent and maintained in shaking condition for 24- 48 hours. After that in hot water bath methanol solvents were evaporated at its boiling temperature. Finally each *G.edulis* methanol residual extract was taken to measure the antioxidant activities.

2.3 Determination of hydrogen peroxide scavenging

The different processed conditions of *G.edulis* sample antioxidant ability were measured by hydrogen peroxide scavenging activity according to the method of (Ruch, et al., 1989). The reaction mixture was been 1 ml of hydrogen peroxide solution (35.4 mM) and different processing conditions of methanol extract of *G.edulis* . Sum of the reaction mixture was 3 ml. at 230 nm hydrogen peroxide absorption was read within 3 min against a blank solution that contained ethanol without hydrogen peroxide. Ascorbic acid was used as reference standard. Experiment was done in six times to each different processed conditions sample. Spectrophotometer (Dr 5000) was used to measure the absorption.

% scavenging of hydrogen peroxide effect=(Ac-As/Ac)*100

where Ac = control absorption, As= absorbance of sample absorption.

(hydrogen peroxide solution in ethanol without sample for used as control).

2.4 Statistical analysis:

Microsoft excel 2010 version and Minitab15 (Minitab 15, 2007) software package were used for statistical analysis. The paired T-test was used for comparison of mean values. Pearson correlation was tested to find significance relationship between samples at different processed conditions and percentage of H_2O_2 scavenging activity. Here P value of <0.05 was considered as statistically significant.

3 Results and Discussion

Table1:Absorbance of different processed *G.edulis* by spectrophotometer (Dr5000)

Samples at concentration 100 µg/ml	Absorbance
Fresh *G.edulis* methanol extract	0.02±0.001
Methanol(Control)	0.128±0.001
L-Ascorbic acid	0.039±0.001
steam *G.edulis*	0.06±0.009
Boiled *G.edulis*	0.01±0.005
Dried *G.edulis*	0.04±0.009
microwaved *G.edulis*	0.023±0.001

Table2: Hydrogen peroxide scavenging activity of different processed *G.edulis*

Samples at concentration 100 µg/ml	%of Hydrogen peroxides scavenging activity
Fresh *G.edulis* methanol extract	84.38
L-Ascorbic acid	69.53
steam *G.edulis*	53.13
Boiled *G.edulis*	92.19
Dried *G.edulis*	68.75
microwaved *G.edulis*	82.03

3.1 Effect of different processing conditions on H_2O_2 radical scavenging activity

Paired T-Test and CI: Absorbance, Hydrogen peroxide scavenging ac

```
Paired T for Absorbance - Hydrogen peroxide scavenging ac

                          N   Mean  StDev  SE Mean
Absorbance                7    0.0    0.0      0.0
Hydrogen peroxide scaven  7   64.3   31.1     11.8
Difference                7  -64.2   31.1     11.8


95% CI for mean difference: (-93.0, -35.4)
T-Test of mean difference = 0 (vs not = 0): T-Value = -5.46  P-Value = 0.002
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Correlations: Absorbance, Hydrogen peroxide scavenging ac

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Pearson correlation of Absorbance and Hydrogen peroxide scavenging ac = -0.988
P-Value = 0.000
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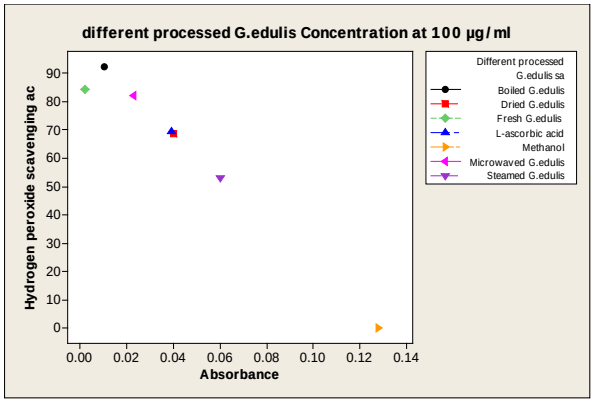
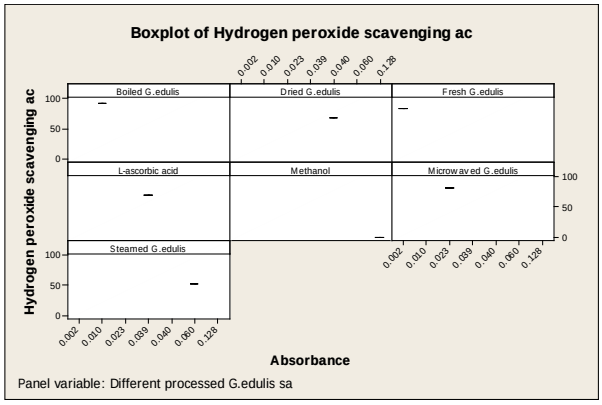
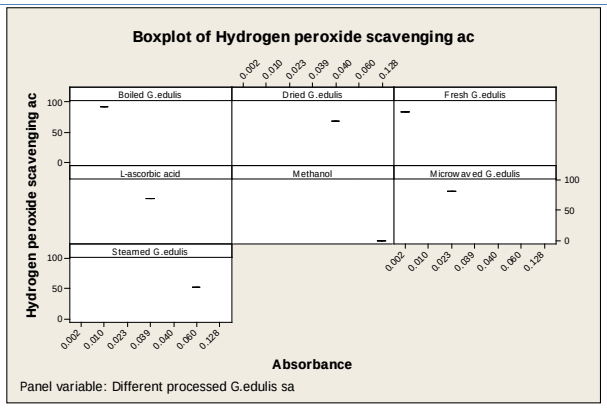
Graph:1different processed *G.edulis* sample Vs absorbance



Graph:2 Hydrogen peroxide scavenging activity Vs Absorbance





Absorbance of different processed *G.edulis* at 230 nm was clearly shown in table 1.H_2O_2 free radical scavenging activity of those samples *G.edulis* results were resulted in table 2. That free radical scavenging activity results were varied between 53.125% to 92.1875% at 100 µg/ml extract and sample boiled at 100 °C being most effective. There were significant differences ($P < 0.05$) for all different processing conditions in H_2O_2 free radical scavenging activity. In heat process, H_2O_2 radical scavenging activity was slightly led to increase from 53.125% to 92.1875% while absorbance led to a significant decrease ($P < 0.05$) in 100 µg/ml extract.

The table 1.1 was shown the H_2O_2 radical scavenging activity that means antioxidant activity were presenced in all processed *G.edulis* sample. Pearson correlation Graph 1 Antioxidant properties of all processed sample were been correlated to its absorbance. That stated antioxidant compounds were presence of those sample. Here electron donations or hydrogen donor was occurred efficiently. In 45 minutes steamed sample, H_2O_2 radical scavenging activity ($P < 0.05$) was recorded very lowest such as 53.125%. In 12 hrs drying pre-treatments H_2O_2 radical scavenging activity was 68.75%. In microwaved, H_2O_2 radical scavenging activity was 82.03125% at 900W. H_2O_2 radical scavenging activity was recorded highest in boiled *G.edulis* sample at 100°C such as 92.1875%. Boiled *G.edulis* was shown highest H_2O_2 radical scavenging activity that revealed boiled *G.edulis* was recorded comparatively highest antioxidant properties than other processed *G.edulis* sample due to leaching of less potent constituents into boiling water however microwaved and steamed samples were not in direct contact with water for such leaching to occur. Antioxidant activity of Boiled *G.edulis* was recorded highly followed by fresh, microwaved, dried and steamed. Steamed *G.edulis* was lowest antioxidant activity than others. Scavenging effects of the steam dried, microwaved, fresh and boiled were increased up to 53.125%, 68.75%, 82.03125%, 84.375% and 92.1875% at 100 µg/ml extracts. In Graph 1&2 There was strong negative correlation between scavenging ability on H_2O_2 radical and absorbance of total antioxidant compounds of *G.edulis* and positive correlation between antioxidant activity and the antioxidant compounds presence in *G.edulis*. Excellent antioxidant effect was screened at boiled one.

4 Discussion

Nearly 150,000 seaweeds were found in the intertidal zones of ocean environment (Amin, 2002). Especially in Asian countries such as Japan, Korea and china are used as most ecologically important macro algae for food. However genus (Gracilariales, Rhodophyta) was an important seaweed group with more than 300 species of which 160 have been known and accepted taxonomically (Layase, 2011). Usually

green, brown and red algae were found in subtropical and tropically which are synthesized bioactive compounds such as primary metabolites with antibiotic ability (Kayatzi, 2012).

Natural antioxidants and anti-properties of marine algae were reported in several research studies (Chew, 2008). The marine algae can grow in harsh environment with combination of oxygen concentration which is the reason formation of strong oxidizing agents and free radicals but they have special mechanisms and compounds for seldom suffer any serious photodynamic damage during metabolism (Matsukawa et al.,1997). Free radicals can cause cancer, cardiovascular and neural disorders Alzheimer's disease in human. Dietary antioxidants especially *G.edulis* foods protect the human body from these types of diseases via trap or inactivation the free radicals. So Antioxidant compounds were used as health protecting factor for reduce the risk for chronic diseases such as cancer and heart diseases. Such antioxidant nutrients protect from free radicals for improving quality of life by prevent onset of degenerative diseases.

Edible seaweeds, fruit, vegetable and whole grains contain main source of natural antioxidants. Most of the antioxidants in a diet derived from plants such natural antioxidants are phenolic acids, phytate, phytoestrogens, carotenes, vitamin C and vitamin E. which were consisted wide variety of physical and chemical properties and high potential to reduce that type of diseases. For example mono- phenols are weak antioxidants and gallates are strong antioxidants. *G.edulis* species have such type of antioxidant compounds. Antioxidant compounds were inactivated free radicals. In biological system, existing oxygen species and highly reactive free radicals were oxidized nucleic acids, DNA, proteins and lipids to cause degenerative diseases (Miller and Rigelhof, et al., 2000). Marine algae especially red algae *G.edulis* contain antioxidant compounds like flavonoids alkaloid and phenol scavenge free radicals such as hydroperoxide or lipid Peroxyl and peroxide for inhibit the oxidative mechanisms that lead to Prevent degenerative diseases. This research belongs to effect of different processing conditions such dried, steam, boiled and microwaved of *G.edulis* on antioxidant ability were investigated.

In recently various methods are used to analyze antioxidant activity such as oxygen radical absorbance capacity (ORAC) assays and enhanced chemiluminescence assays. These methods are needed special equipment and good technical skills. ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation is used to analyze relative radical-scavenging activities of phenolics and flavonoids through their Prior. In the presence of the antioxidant Thiobarbituric acid-reactive-substances (TBARS) (1) or malondialdehyde (MDA) assays to estimate the peroxidation of lipids in membrane and biological systems. Which is time consuming method due to oxidation of a Substrate depend on pressure matrix and temperature and may not be practical when large numbers of samples are involved. Results will be varied depending on the specific free radical being used as a reactant in different antioxidant assay. Hydrogen peroxide assay measure the radical scavenging activity of antioxidants presence in sample against free radicals like H_2O_2 radical, hydroxyl radical ($OH^{\cdot}$), superoxide anion radical ($O_2^{\cdot}$), peroxyl radical ($ROO^{\cdot}$). Antioxidant activity measure by using trap or inactivate free radical which is relatively straightforward to perform antioxidant ability. H_2O_2 scavenging activity gives overall antioxidant capacity of sample for understand the functional properties of food which method is rapid, simple and inexpensive best method. The H_2O_2 scavenging activity of *G.edulis* methanol extract was tested by using H_2O_2free radical unlike superoixide anion and hydroxyl radical because such are laboratory generating free radical. *G.edulis* methanol extracts were free radical scavenger or free radical inhibitors and it was used as primary antioxidants. *G.edulis* was shown scavenging activity in H_2O_2 radical due to presence of active substances

including flavanols, pigments, alkaloids and some other phenolic compounds. Here electron donating ability was scavenged H_2O_2 as possible mechanisms for their antioxidant ability (Wright2 mechanism). The inhibition rate or scavenging activity was varied depend on the processing conditions. Antioxidants in algal foods are water soluble, insoluble, fat soluble which bound to cell wall but not free available to react with H_2O_2. However different processed *G.edulis* samples were reacted with different rates. The antioxidant activity was expressed in absorbance of sample and its control at the endpoint. During the period H_2O_2 react with *G.edulis* methanolic extract. Their antioxidant activity was increased with the time and stops at certain time. Various antioxidant analyses are limited to those compounds soluble in selected solvent. Methanol was the best extraction solvent (Kolanginathan., 2009). Whole sample was allowed to react with H_2O_2 in sufficient time because reaction was slowly in week antioxidant.

While consider modulation of free radicals by natural antioxidants there are two type of antioxidants such as enzymatic antioxidants and nonenzymatic antioxidants which were modulated the free radical reactions. Enzymatic antioxidant mechanisms protect the human body from ROS (Koruk, et al.,2004). Antioxidant enzymes reduce H_2O_2 and lipid hydroperoxide level, which are used to maintain structure and function of cell membranes and lipid hydroperoxide.Examples of the enzymatic antioxidants (Fig.3, are CAT, GSHPx, SOD, and peroxiredoxin I–IV (I–IV).

$$2H_2O_2 \xrightarrow{\text{(catalase)}} 2H_2O + O_2$$

$$ROOH + 2GSH \xrightarrow{\text{(GSHPx)}} ROH + GSSG$$

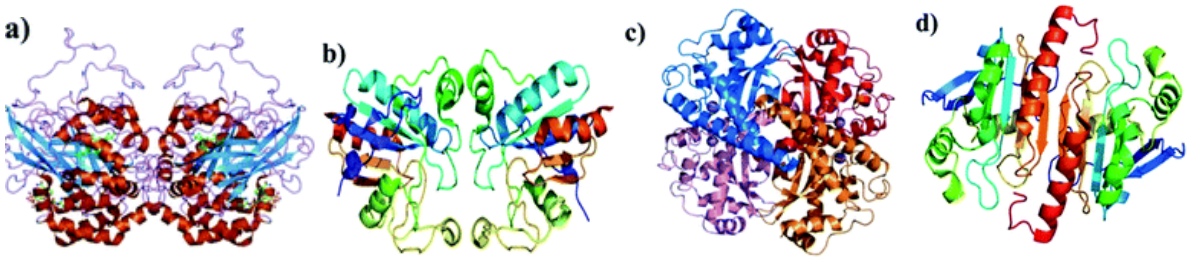
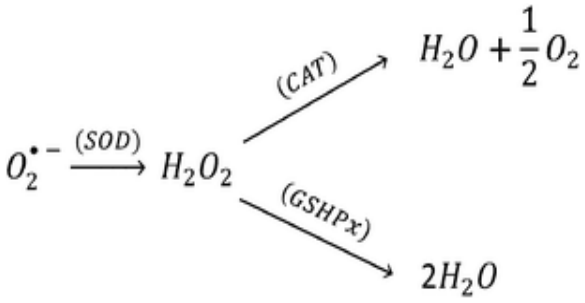


Figure. 3 (a) CAT, (b) GSHPx, (c) SOD, and Prx-I. Nimse, S.B and Pal, D,_5 online Article, image, viewed 2015, http://pubs.rsc.org/en/journals/journal/RA?issueid=RA005035

Table 3 Enzymatic antioxidants, their cellular locations and the reactions they carry out

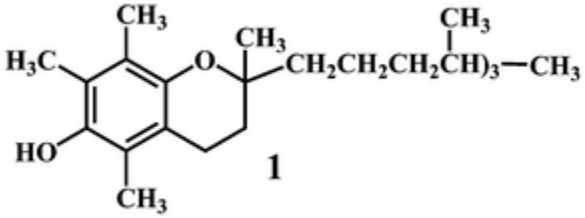
Substrate	Enzymatic antioxidant	Cellular location	Reaction
H_2O_2	CAT	Peroxisomes cytosol	$2H_2O_2 \rightarrow O_2 + H_2O$
H_2O_2	GSHPx	Cytosol	$H_2O_2 + GSH \rightarrow GSSG + H_2O$
$O_2^{\cdot -}$	Mn/Cu/Zn SOD	Mitochondrial matrix (Mn SOD) cytosol (Cu/Zn SOD)	$O_2^{\cdot -} \rightarrow H_2O_2$
H_2O_2	Prx–I	Cytosol	$H_2O_2 + TrxS_2 \rightarrow Trx(SH)_2 + H_2O$

In the presence of metal ion co factors, $O_2^{\cdot -}$ catalytically convert into H_2O_2 and Oxygen while SOD's located in the mitochondria and cytosol (Gough et al., 2011). In the presence of CAT enzyme in the peroxisome H_2O_2 convert into to water and oxygen (Stone, et al., 2006). GSHPx is found in human tissue and cytoplasm as extracellular component which has strong activity against hydroperoxides, fatty acid and H_2O_2 which enzyme convert the H_2O_2 into the water(Arthur, et al., 2000). Peroxyredoxin enzyme was catalyzed the reduction of peroxynitrite ($ONOO^-$), H_2O_2 and organic hydroperoxides.The antioxidant enzymes consist a vital role for prevention of oxidative damage. SOD, GSHPx and CAT were given synergistic effect in the scavenging of $O_2^{\cdot -}$ (Valko, et al., 2007). **Scheme 1**



Scheme1: Radical scavenging activity of SOD, GSHPx and CAT

Most of biochemistry research article illustrate enzymatic antioxidants and their mechanisms (Mates, et al.,2000). This paper was focused only nonenzymatic antioxidants which are two types synthetic antioxidants and natural antioxidants. However this research paper was focused on natural antioxidants.



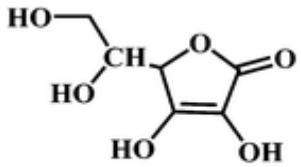
Picture:1 Vitamin E 1

Efficient lipid soluble antioxidant Vitamin E(α-tocopherol) chemical structure was shown in picture1. This is worked as chain breaker during lipid peroxidation in cell membranes. Intercept lipid peroxyl radicals

(LOO˙) and to end up the lipid peroxidation chain reactions were done by this antioxidant (Morliere, et al., 2012).

$$LOO^{\cdot} + \alpha\text{-tocopherol–OH} \rightarrow LOOH + \alpha\text{-tocopherol–O}^{\cdot}$$

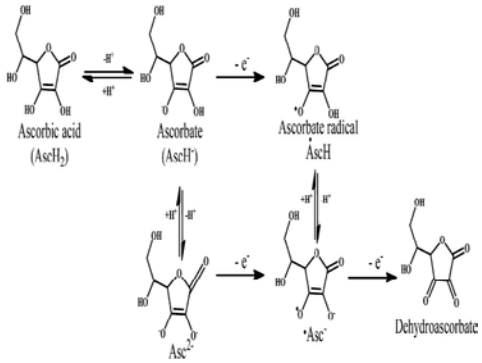
Tocopheroxyl radical is insufficiently reactive to initiate lipid peroxidation itself which is relatively stable good antioxidants (Witting, et al.,1997).Vitamin E scavenge lipid peroxyl radicals in vivo as well as in vitro systems. This antioxidant in vivo is not an efficient scavenger to alkoxyl radicals (˙OR) and˙OH (Morliere, et al., 2012).



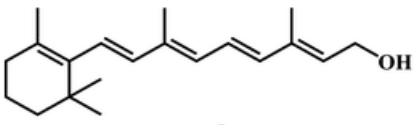
2

Picture: 2 Vitamin C 2,

Vitamin C is ascorbic acid and used as positive control. This water soluble antioxidant has ability to scavenge H_2O_2 free radical and also activates vitamin E in cell membranes in combination with compounds or GSH ability to donate reducing equivalents (Oh, et al., 2010). by giving an electron to the lipid radical in order to terminate the lipid peroxidation chain reaction, vitamin C changes to the ascorbate radical. Pairs of ascorbate radicals react rapidly to form one molecule of ascorbate and one molecule of dehydroascorbate. dehydroascorbate is not having antioxidant properties. Dehydroascorbate change into ascorbate by addition of two electrons. Finally in presence of oxidoreductase, two electrons have been added to the dehydroascorbate. **Scheme 4** (Oh, et al., 2010).



Scheme 4 Mechanism of radical scavenging activity of ascorbic acid

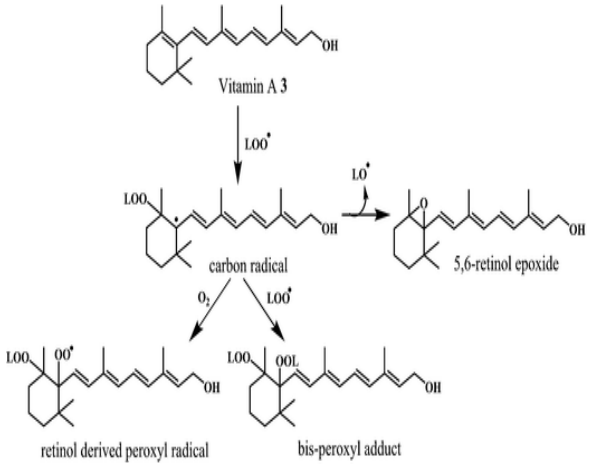


3

Picture: 3 Vitamin A 3.

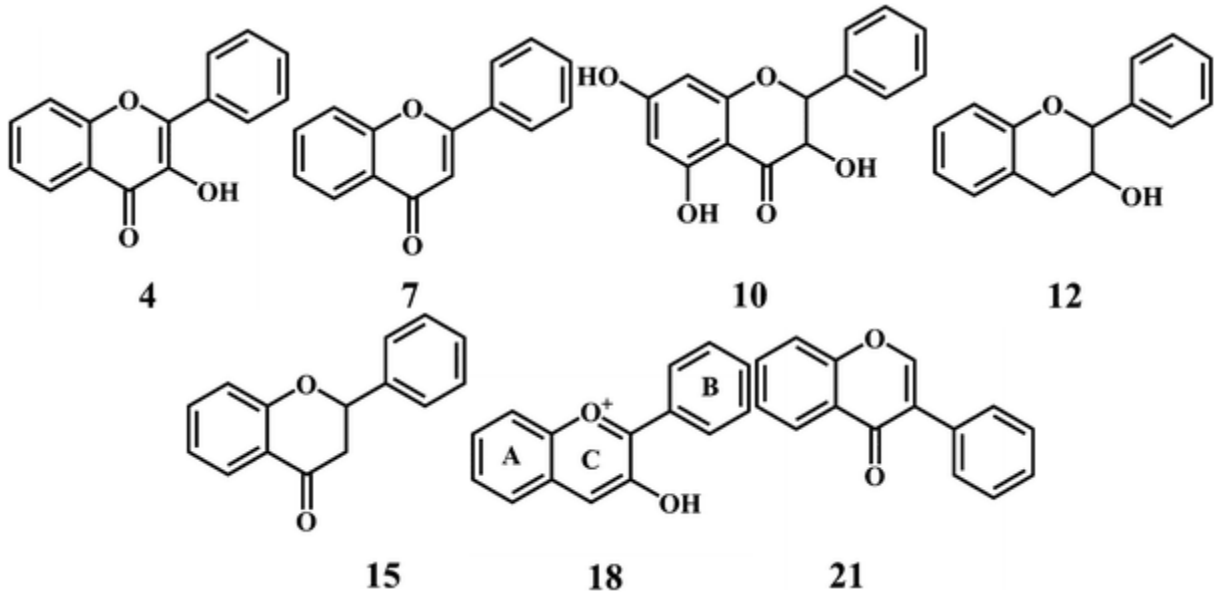
Antioxidant potential of vitamin A 3 protects lipids against rancidity. (Monaghan and Schmitt,1932).While consider structural and metabolic characteristics Vitamin A is relation to heart diseases(Tesoriere, et

al.,1997). This has vital antioxidant contribution in protecting low density lipid against copper-stimulated oxidation. (Scheme5). (Tesoriere, et al., 1997).



Scheme 5 Mechanism of radical scavenging activity of vitamin A

Bioflavonoids

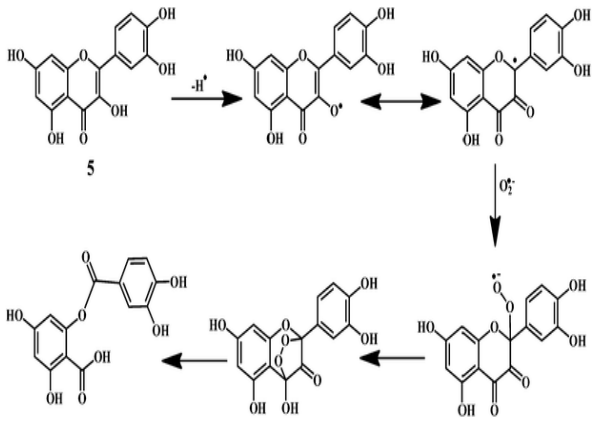


Picture:4 Flavonol 4, Picture:5 flavone 7 , Picture:6 flavonolols 10, Picture:7 flavan-3-ols 12, Picture:8 flavonone 15, Picture:9 anthocyanidin 18, Picture:10 isoflavone 21

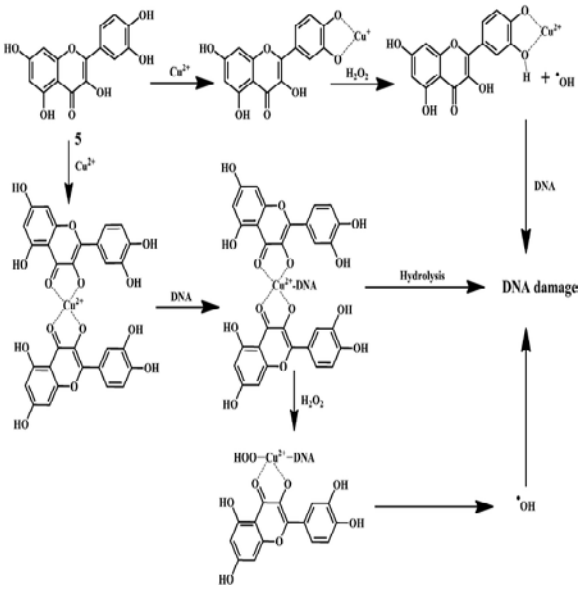
Bioflavonoids are found in *G.edulis*. This antioxidant is found naturally as benzo-γ-pyran derivatives which have strong free radical-scavenging activity (Armida, et al.,2005).These compounds are widely distributed in Vegetables as well as fruits and having protective mechanisms against DNA damage induced by the hydroxyl radicals (Nijveldt, et al., 2001). chelating metal ions especially iron and copper are involved with that protective mechanisms as prevent the formation of ROS (Nijveldt, et al., 2001).

Flavanol compound is one of Quercetin 5 that is protected the DNA from oxidative damage resulting from attach of $O_2^{\cdot -}$ $^{\cdot}OH$ and H_2O_2 on the DNA oligonucleotides. Scheme 6 (Jun, et al., 2007). Quercetin has shown opposite effects on DNA damage in presence of cupric ion. Scheme7 (Jun T et al.,2007). Quercetin

enhances the damage to DNA by ROS in its high concentration (≥25 µM). Quercetin gives a protective role in its low concentration (≤25 µM). Therefore it is very important matter to consider chelating metal ions, such as copper or iron in the quercetin and other bioflavonoids activity.

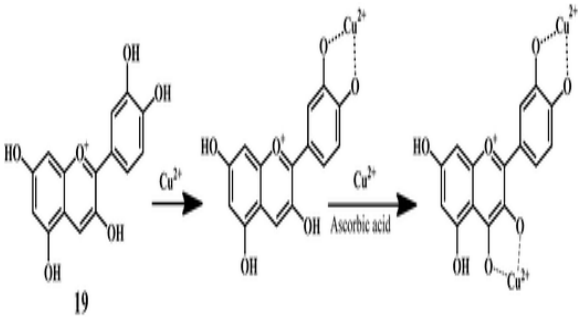


Scheme: 6 Mechanism of superoxide anion radical scavenging activity of quercetin

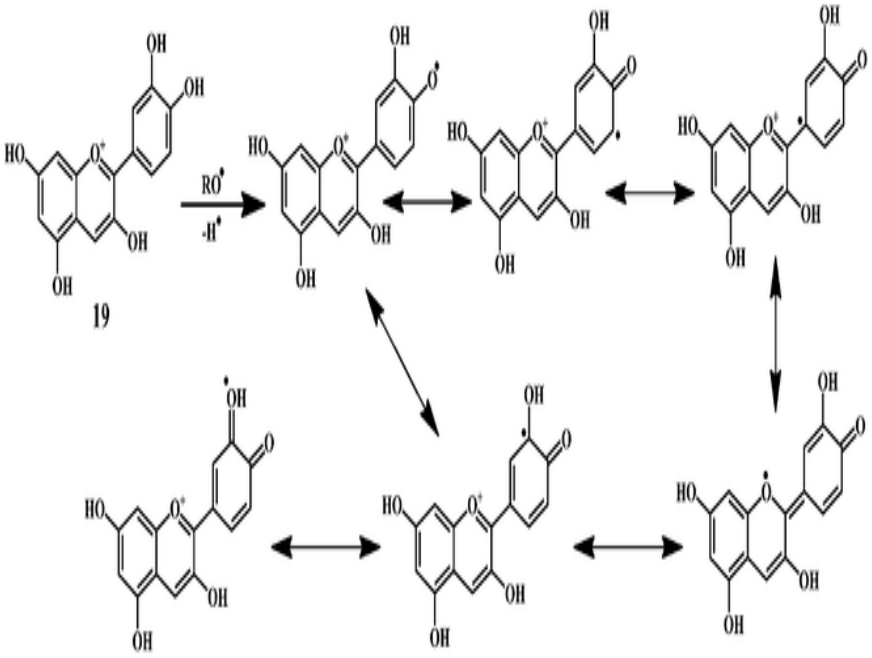


Scheme: 7 Mechanism of DNA damage induced by quercetin copper complex.

Antioxidant activity of pigment of Rhodophyta is Anthocynidine which is under class of flavonoids. Its effectives depend on inhibition of the lipid oxidation and its metal ion-chelating activity (Scheme 8) and also free-radical scavenging activity (Scheme 9). Radical-scavenging activity of anthocynidines determine by its three structural group such are ortho-dihydroxy structure in the B-ring, double bond in conjugation and 4-oxofunction in the C-ring (Miguel, el al.,2011). Flavonoids form different complexes with metal ions by hydroxyl groups in ortho position in the B-ring or using the 3- or 5-hydroxyl and 4-ketosubstituents. (Miguel, el al., 2011).



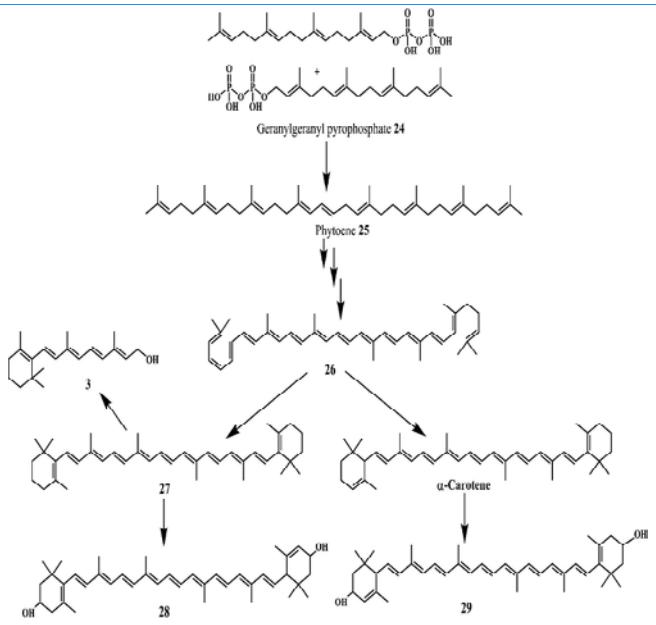
Scheme: 8 Metal ion (Cu^{2+}) chelating activity of anthocynidine (cynidin 19)



Scheme: 9 Mechanism of radical scavenging activity of cynidin

While consider Scheme 9, Anthocynidins donate an electron to a free radical of OH group attach in phenol ring for free radical inactivation (Acker, et al., 1996). In this process polyphenolic reducing agent changes to an aroxyl radical which is more stability due to its resonance effect rather than free radical reduced. Termination of damaging oxidative chain reactions takes place in overall reactions.

Carotenoid is one of the pigments present in Rhodophyta. This antioxidant is lipid soluble phytonutrients. β-carotene 25 and Lycopene 24 are main carotenoids among its group (Mueller, et al., 2011). The synthesis of carotenoids 26–29 from phytoene 25 was shown in Scheme 10. Carotenoids scavenge ROS lowerly than peroxyl radicals. Peroxyl radicals damage the lipids presence in cell wall in the lipid peroxidation process. This damage can be prevented by Scavenging of peroxyl radicals (Mueller, et al., 2011). In the carotenoids, long unsaturated alkyl chains form them highly lipophilic. Carotenoids react with peroxyl radicals to form resonance stabilized carbon-centered radical adducts for peroxyl radicals deactivation.



Scheme: 10 Biosynthetic pathway for the synthesis of carotenoids 26–29

Lycopene 24 is also found in vegetables and fruits. This has prominent antioxidant activity. Lycopene Conjugated double bonds donate the singlet oxygen quenching ability. This ability is comparatively higher than α-tocopherol 1 or β-carotene 25(Erhardt, et al., 2003). β-Carotene contain antioxidant ability due to its interaction with biological membranes and chemical structure (Erhardt, et al.,2003). (Z)-isomers of the β-carotene give antioxidant activity. In addition β-carotene-15, 15'-dioxygenase catalyzed cleavage gives two molecules of vitamin A from β-carotene.

The table 1 was shown the absorbance of all different processed *G.edulis*. These results revealed all processed *G.edulis* sample were shown prominent antioxidant activity. In minitab-15 statistical software package, the absorbance, inhibition or hydrogen peroxide scavenging activity depend on the processing conditions of the *G.edulis* methanol extract was clearly resulted by Paired T-Test. Paired T-Test statistical test did tell about the difference between the different processing conditions of the methanol extract of the *G.edulis* ($p<0.05$) and antioxidant activity. Which two factors were not independent factors such are depend on each other. While Hydrogen peroxide absorbance was decreased, the percentage scavenging of hydrogen peroxide or inhibition was increased and antioxidant activity was also increased. The methanolic extracts of the *G.edulis* was prepared keeping methanol as a standard. In Graph 1Pearson correlation was shown Antioxidant properties of all processed sample were been correlated to its absorbance. That means antioxidant compounds were presence of those sample. Here a hydrogen donor or electron donation was occurred efficiently. All different processed *G.edulis* methanol extract samples antioxidant properties were compared to the L-ascorbic acid which used as positive control. In the Table 2, boiled *G.edulis* sample was recorded comparatively highest antioxidant properties than other processed *G.edulis* sample due to less leaching seaweeds phytochemical constitutions. Antioxidant activity of this *G.edulis* depends on its presence of bioactive compounds. Absorbance was highly in steamed followed by dried, microwaved, fresh and boiled *G.edulis* respectively due to presence of low amount bioactive antioxidant compounds basis. Hence H_2O_2 scavenging activity or antioxidant activity was

highly in boiled *G.edulis*, followed by fresh, microwaved and dried respectively. L-ascorbic acid was used as Positive control and its antioxidant activity was higher than dried and steamed but relatively lowers than microwaved fresh and boiled *G.edulis*. Antioxidant activity of Boiled *G.edulis* was recorded higher activity than other processed *G.edulis* followed by fresh, microwaved, dried and steamed respectively. Steamed *G.edulis* was lowest antioxidant activity than others. Scavenging effects of the steam, dried, microwaved, fresh and boiled were increased up to 53.125%, 68.75%, 82.03125%, 84.375% and 92.1875% at 100 µg/ml *G.edulis* samples methanol extract. In Graph2, There was strong negative correlation between scavenging ability on H_2O_2 radical and absorbance of total antioxidant compounds of different processed *G.edulis* and positive correlation between antioxidant activity and the antioxidant compounds presence in different processed *G.edulis*. Excellent antioxidant effect was recorded at boiled one.

5 Conclusion

This research paper finding was indicated that the processing conditions significantly influenced the antioxidant activity of *G.edulis* in order to find the most potent functional processed *G.edulis*. Antioxidant activity of different processed *G.edulis* was a better knowledge of how these processing conditions affects the antioxidant compounds. Processing parameters such as heat was influenced the loss of health related antioxidant compounds and phytochemicals. Since from ancient times people may be known heat processing was invariably leads to a loss of antioxidant properties a compromise must be reached between nutrition and palatability. But this present research study revealed that a boiling of *G.edulis* was reduced processing time and to less leaching of phytochemicals or antioxidant compound. The antioxidant activity of different processed *G.edulis* was determined rapidly and accurately by quick method of H_2O_2 scavenging activity, which method was conducted successfully for algae with methanolic extraction. While scavenging the H_2O_2 free radical by phytochemical in the *G.edulis* methanolic extract, antioxidant activity was measured by absorbance of spectrophotometer. Antioxidants were been varied due relative antioxidant content such as flavanols, biflavanoids, pigments, fiber, mineral and vitamins. The reason behind the antioxidant of boiled *G.edulis* has greater potential to reduce free radical in the human body Where the expose high antioxidant in *G.edulis* caused removal or reduction of free radical in body highly. Therefore these findings were concluded antioxidant content and basic nutritional information of the different processed *G.edulis* samples.

Furthermore the antioxidant activity was higher in boiled *G.edulis* followed by dried, microwaved, fresh, dried and steam respectively. Finally Boiled *G.edulis* sample was recorded prominent antioxidant activity due to leaching of less antioxidant compounds. From this research results boiled *G.edulis* was a good antioxidant activity rather than other processed *G.edulis* and this processing method was recommended. In future these research findings were used for functional product development incorporation of boiled G*. edulis* into bakery Products and supported to predict new type of food production in the business market in Sri Lanka. Thus the consumption of *G.edulis* food products may result good health promoting benefits either therapy or human nutrition to save the human life from degenerative diseases.

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