International Journal of Research
Available at https://edupediapublications.org/journals
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 04 Issue 09
August 2017

Antioxidant activity of *Gracilaria edulis*(Rhodophyseae) in Sri Lanka

G.Umakanthan*[1], P.Vinobaba[2], K. Radampola[3]
[1-3]Department Fisheries and Aquaculture, University of Ruhuna
[2]Department of Zoology, Eastern University, Sri Lanka
ghumakanth@gmail.com

ABSTRACT

In recently marine seaweeds especially Gracilaria edulis are used to produce new drugs and healthy delicious low calorie foods such as jelly, jams, puddings, and soup which are known as primary source of secondary metabolite and to be used as natural antioxidants and antimicrobials. The purpose of this study was determined the antioxidant activity of G.edulis. Methanol extract of G.edulis were prepared in different concentrations such as 5, 10, 15, 20, 25 and 30 µg/ml. The antioxidant activity of G.edulis was determined by hydrogen peroxide scavenging assays in absorbance at 230 nm. In 30µg/ml methanol extract of G.edulis was shown the highest total antioxidant activity ($p<0.05$) while compared with other G.edulis sample concentration. Steady state antioxidant and free radical scavenging activities of methanol extract of G.edulis were exhibited at its 15ug/ml. In IBM SPSS package 2010, chi squared test and Paired T-Test were clearly illustrated the absorbance, inhibition or percentage scavenging of hydrogen peroxide depend on the concentration of the G.edulis methanol extract. These two statistical tests did tell about the difference between the concentration of the methanol extract of the G.edulis ($p<0.05$) and antioxidant activity. While methanol extract of G.edulis concentration increase, Hydrogen peroxide absorbance was decreased, the inhibition or percentage scavenging of hydrogen peroxide was increased and antioxidant activity was also increased. The antioxidant activity of G.edulis methanol extract were determined and compared with other aromatic compounds such were 2, 4 dinitro phenyl hydrazine and Aniline derivatives. H_2O_2 Scavenging activity assay was used to analyze the antioxidant activity to all investigated series concentration compounds. Aniline compounds were more active than other tested aromatic compounds. Antioxidant activity of these tested compounds depends on presence of active groups and its Position and number. OH and NH_2 was highly active their presence in ortho followed by para and meta. NH_2 electron donor substitution group transfer its electron to free radical source becoming itself a radical cation. Therefore it gives positive effect to H_2O_2 scavenging activity and antioxidant activity and had lower IP value. NO_2 groups are electron withdrawing group which groups were negative effects to H_2O_2 scavenging activity antioxidant activity. Absorbance were highly in 2, 4 dinitro aniline followed by 2, 4 dinitro phenyl hydrazine, aniline and G.edulis due to the H_2O_2 scavenging activity. Hence antioxidant activity or H_2O_2 scavenging activity was highly in G.edulis, followed by aniline, 2,4 dinitro phenyl hydrazine and 2,4 dinitro aniline. Finally G.edulis sample was recorded prominent antioxidant activity. This study is used in future for product development incorporation of dried seaweed G. edulis into bakery Products.

Keywords: Marine Sea weed, H_2O_2, Solvent Extracts, Antioxidant Compounds.

INTRODUCTION

Seaweeds are known as Marine algae which are multicellular, macrothalic and macroscopic marine benthic algae. Researchers found probably 45000 seaweed species in the world. That are classified into brown algae, red algae, and green algae in marine ecosystem.(Amin 2002).which are found in the deep shallow and intertidal areas up to 180m depth which also grown in lagoons estuaries and backwater on the plant and other hard solid material substrate typically in pebbles, rocks, shells, deep corals. Marine algae are having various essential biological activities due to their bioactive compounds. (Bouhlar et al., 2011, Kayatzi, 2012). These bioactive compounds are able to synthesize secondary metabolites. Green, brown and Red algae derived compounds show antiviral antimicrobial antifungal and antioxidant activities (Yuan *et al.*, 2005; Bansemir *et al.*, 2006; Chew *et al.*, 2008). Human consuming seaweeds have been reduced to the prostrate and breast cancer in china and Japan than the other countries. (skiboda.C, 2004) which are main source of more than 600 secondary metabolites. Most of the edible seaweed species give wide range of biological activities such as antihelminthic, antimicrobial, antiinflamatory and neurotoxin properties. (Itoc & Aorik.,1989, Pisani et al., 1990. In red seaweed especially *Gracilaria edulis* contain Primary and secondary metabolities. (Layse, 2011).

In Combination of high oxygen concentration and light environment seaweeds can be able to produce strong oxidizing agents and free radicals. This can be reason for serious photodynamic damage during metabolism. Bioactive compounds of edible seaweeds give protective mechanisms against free radicals. (Matsukawa *et al.*, 1997). Peroxyl radicals, hydroxyl and superoxide are reactive oxygen species (ROS) which give extensive oxidative damage and cause age related degenerative conditions, cancer and other diseases in human by endogenous factors. (Reaven and Witzum, 1996; Aruoma, 1999). Most of micro nutrients commonly found in Green, brown and red algae are antioxidants (Chanda.S.,2010). Phenolic compounds of the seaweeds structure is benzene ring substituted by at least one hydroxyl group. (Manach *et al.*, 2004; Duan *et al.*, 2006). These Phenolic compounds act as antioxidant activities by improving the antioxidant endogenous system preventing radical formation and chelating metal ions. (Al-Azzawie and Mohamed-Saiel, 2006). Recently diverse group of poly phenols such are flavonols, flavones, flavanones, flavononols, flavan-3-ols, chalcones, phenolic acids, tocopherols, tannins and lignins are the source natural antimicrobial and antioxidant to reduce the use of BHT and BHA. (Shukla *et al.*, 1997). Generally natural antioxidants react with free radicals to reduce oxidative damage (Akoh and Min, 1997). These natural antioxidants can lead to increase the shelf life of foods and protect human body from oxidative damage. (Chandini *et al.*, 2008). Tannin is one of the natural phenolic metabolite widespread in terrestrial and marine plants which compound is categorized into hydrolysable and condensed compounds. (Haslam, 1989; Waterman and Mole, 1994). But Phlorotannins of Tannin compounds group derived from marine plants possess antioxidant activity. Phlorotannins eight interconnected rings molecular structure is unique.

This tannin group formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine algae (Ragan and Glombitza, 1986; Waterman and Mole, 1994; Arnold and Targett, 1998).

Phytoestrogens phyruvate, Carotenoids, vitamin E, Vitamin C, and phenolic acid are antioxidant compounds which protect the body from Oxidative stress. Oxidative stress can cause inflammation aging cancer ischemic injury alzheimerss and Parkinson disease. Oxidative stress generate by one or more unpaired electrons in an outer orbital free radical which are reactive oxygen and nitrogen species (Meenakshi et al., 2009). Antioxidants neutralize

the free radical from a variety of sources. Normally free radicals and oxygen species oxidize DNA, protein nucleic acid and lipid. (Vadlapadi.V et al., 2010). *G.edulis* contain antioxidants such as flavonoid, phenolic acid and poly phenol inhibit oxidative stress that lead to degenerative similar diseases. Rinelhof et al 2000).

Phenolic compounds have defense mechanisms against microbes and other types of environmental stress, such as excessive light and wounding (Harbourne, 1994; Herrmann, 1989; Wallace and Fry, 1994). Especially Flavonoids are the largest group of phenolic compounds consist antioxidant and free radical scavenging activities (Kahkonen *et al*., 1999). Flavonols were showed antioxidant activity in the quantum mechanical study because of its radicals planar conformation gives extended electronic delocalication between adjacent rings (Russo et al., 2000). Biochemical compositions of *G.edulis* contain flavanol. (Umakanthan et al., 2015). Hence *G.edulis* can be resulted antioxidant acitivity definitly.
The Presence, Kind and Position of various active groups are possible to form structure activity relationship such are scavenging and antioxidant activities. In the presence of free radical antioxidants can result 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 of ArOH loses its hydrogen atom and become a radical where antioxidant activity evaluates by OH bond dissociation energy. This mechanism is more favorable to illustrate the DPPH assay results for antioxidant activity. Here OH bond dissociation energy inversely proportional to DPPH scavenging activity and antioxidant activity. (Wright et al., 2001). For an example, lower bond energies or weaker OH bond the easier will be the reaction of free radical inactivation. OH bond has weaker bond dissociation energy so it can be easily broken to give H atom. This H atom reacts with free radical for free radical inactivation.
The second mechanism is more favorable to illustrate H_2O_2 scavenging activity. ArOH antioxidant gives an electron to form radical cation. Here electron donor ability proportional to H_2O_2 scavenging or antioxidant activity. (Wright et al., 2001).

There are several analytical methods to evaluate the radical scavenging activity of antioxidants or inhibition activity or antioxidant activity by oxidation, inhibition rate, the % of the reagents used against free radicals like the hydroxyl radical(OH), superoxide anion radical(O2), 1,1-diphenyl-2-picrylhydrazyl(DPPH), perroxyl radical(ROO) stylet oxygen gunters, metal chelators and hydrogen donating compounds.(Amin and Hong 2002). In another method, antioxidant activity evaluate by primary radicals are reduced to non-radical chemical compounds into oxidize antioxidant radicals by donating hydrogen radicals.(Jadhav et al 1995,Yamaguchi et al 1998, Hwang .Pai.Aa et al 2010 and devi K.P et al 2008). Most of the science researchers were illustrated on the antioxidant activity of seaweed varieties. (Gonzalez del Val *et al.*, 2001; Ganesan *et al.*, 2008; Plaza *et al.*,2009). However, research reports on the antioxidant of seaweed extracts from Sri Lanka particularly widespread of red seaweed *G.edulis* in Kalpitiya, Puttalam are very limited. The purpose of this study was revealed to investigate the antioxidant activity of *G.edulis* and compared with other aromatic compounds such were 2,4 dinitro phenyl hydrazine and aniline derivatives by Hydrogen peroxide scavenging activity.

MATERIALS AND METHODOLOGY

Sample collection:

The *G.edulis* samples were collected from Kalpitiya, Puttalam and transported to the lab in 0°C by keeping in an insulated box for determination of antioxidant activities. The

specimens were identified at Department of Zoology, Eastern University, Sri Lanka.

Preparation of methanol residual extracts of *G.edulis*

The *G.edulis* samples were rinsed thoroughly in fresh water and then distilled water to remove epiphytes and other dirt particles. That was shade dried and blended into fine powder. Then 10grams of that powder was mixed with 100ml of methanol solvent and kept in shaking condition for 24- 48 hours. After that solvents were evaporated in hot water bath at its boiling temperature. The methanol residual extract of *G.edulis* was used to determine the antioxidant activities.

Determination of hydrogen peroxide scavenging

The antioxidant ability of tested compounds by scavenge hydrogen peroxide was determined 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 concentrations of methanol extract of *G.edulis* and tested compounds (from 5µg/ml to 30µg/ml). Total volume of the reaction mixture was 3 ml. Absorption of hydrogen peroxide at 230 nm was determined within 3 min against a blank solution that contained tested compound in ethanol without hydrogen peroxide. Reference standard compound being used was ascorbic acid and experiment was done in six times for each sample. The absorption was measured by using spectrophotometer (Dr 5000).(Picture:1)

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

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

(hydrogen peroxide solution in ethanol without sample).

Statistical analysis:

Statistical analysis was done using Statistical package for social sciences (SPSS 19, 2010) version software package on the data. The ANOVA followed by turkey test was used for comparison of mean values. The paired sample test and chi-squared test to find significance relationship between concentration of sample and percentage of H_2O_2 scavenging activity. P value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Picture 1: Different concentration of methanol extract of *G.edulis* and Aromatic tested compounds preparation and measurements of their absorbance by spectrophotometer (Dr5000)

Table:1 Absorbance of different sample by spectrophotometer (Dr5000)

Concentration µg/ml	*G.edulis* mthanol extract absorbance of sample by spectrometer	Absorbance of Methanol (control)	Absorbance of L-Ascorbic acid	Absorbance of 2,4 Dinitro phenyl hydrzine	Absorbance of Aniline	Absorbance of 2,4 Dinitro Aniline
5	0.0245±0.001	0.244±0.001	0.238±0.001	0.037±0.001	0.035±0.001	0.04±0.001
10	0.0243±0.001	0.242±0.001	0.172±0.001	0.037±0.001	0.034±0.001	0.038±0.001

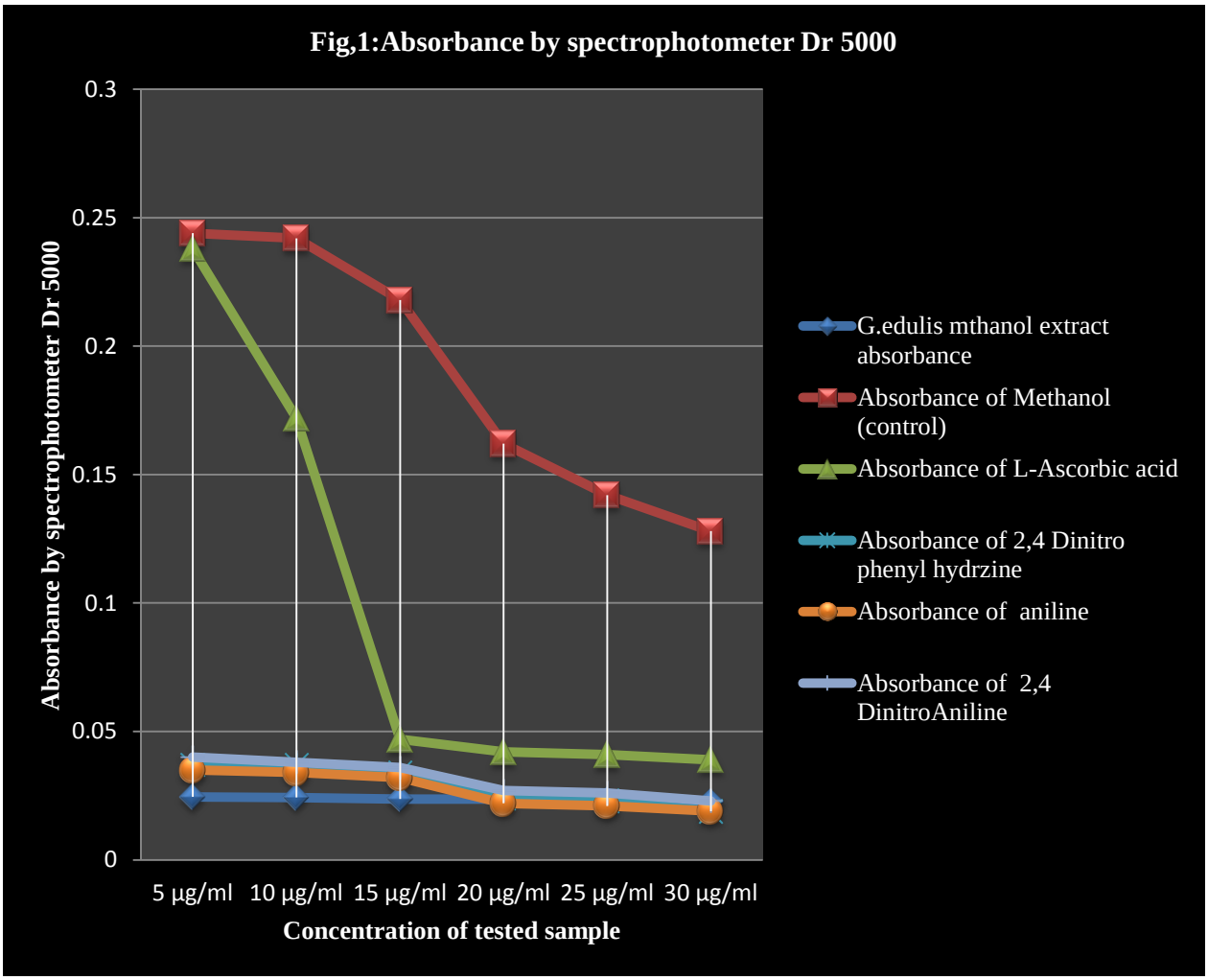
15	0.0237±0.002	0.218±0.001	0.047±0.001	0.034±0.001	0.032±0.001	0.036±0.001
20	0.0237±0.002	0.162±0.001	0.042±0.001	0.024±0.001	0.022±0.001	0.027±0.001
25	0.0233±0.002	0.142±0.001	0.041±0.001	0.023±0.001	0.021±0.001	0.026±0.001
30	0.0228±0.000	0.128±0.001	0.039±0.001	0.019±0.001	0.019±0.001	0.023±0.001

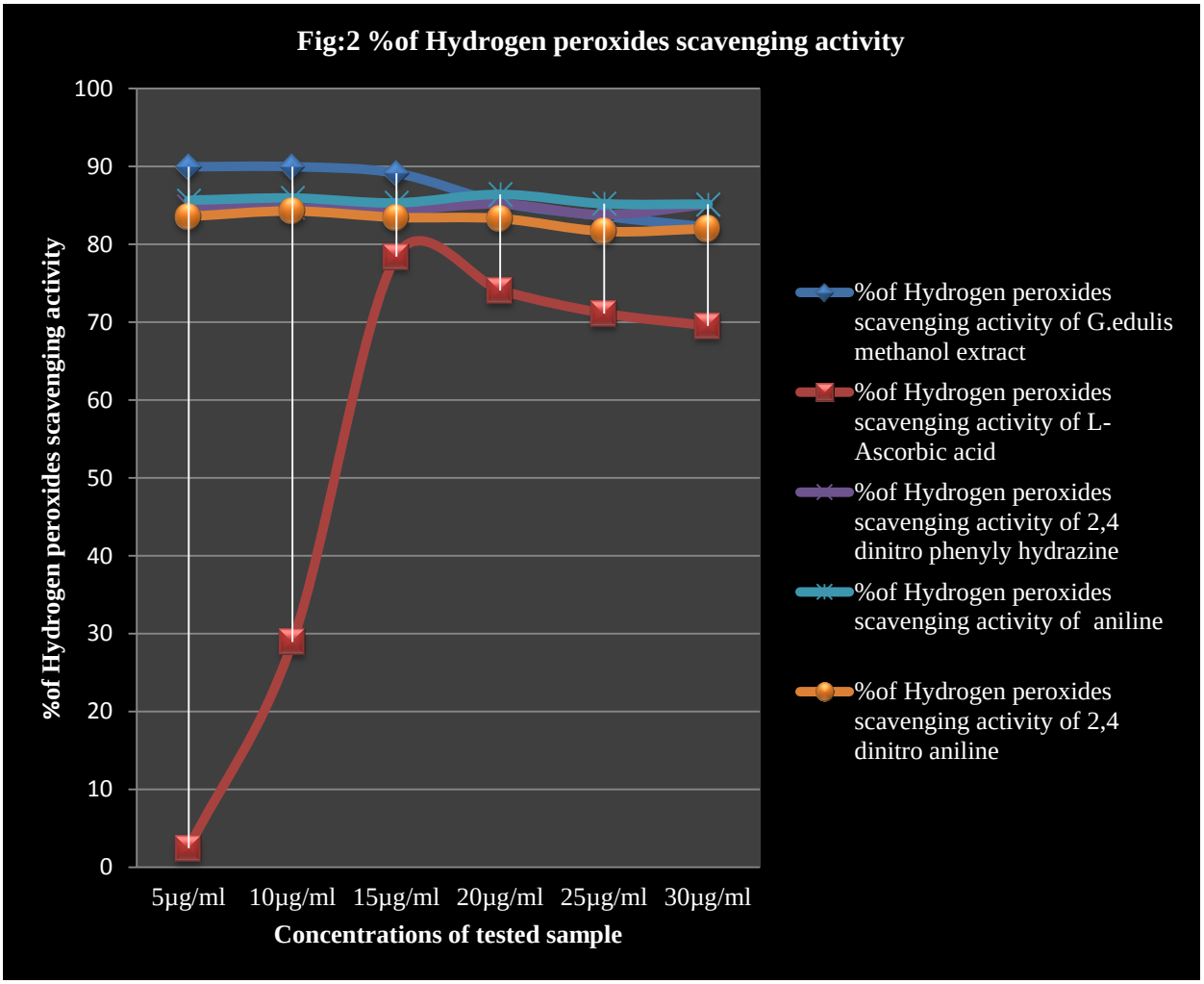
Mean value ± standard deviation

Table:2 The presence of antioxidant activity was shown in the results of H_2O_2 reduction method

Concentration µg/ml	%of Hydrogen peroxides scavenging activity of G.edulis methanol extract	%of Hydrogen peroxides scavenging activity of L-Ascorbic acid	%of Hydrogen peroxides scavenging activity of 2,4 Dinitro phenyly hydrazine	%of Hydrogen peroxides scavenging activity of aniline	%of Hydrogen peroxides scavenging activity of 2,4 Dinitro Aniline
5	89.95902	2.459016	84.83607	85.65574	83.60656
10	89.95868	28.92562	84.71074	85.95041	84.29752
15	89.12844	78.44037	84.40367	85.3211	83.48624
20	85.37037	74.07407	85.18519	86.41975	83.33333
25	83.59155	71.12676	83.80282	85.21127	81.69014
30	82.1875	69.53125	85.15625	85.15625	82.03125

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 04 Issue 09
August 2017





Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Concentration µg/ml - G.edulis mthanol extract absorbance of sample by spectrometer	17.4762833	9.3547582	3.8190640	7.6590667	27.2935000	4.576	5	.006

Pair 2	Concentration µg/ml - %of Hydrogen peroxides scavenging activity of G.edulis methanol extract	-69.1992590	12.6887014	5.1801406	-82.5152344	-55.8832836	-13.359	5	.000
Pair 3	G.edulis mthanol extract absorbance of sample by spectrometer - %of Hydrogen peroxides scavenging activity of G.edulis methanol extract	-86.6755424	3.4325149	1.4013183	-90.2777458	-83.0733389	-61.853	5	.000
Pair 4	Concentration µg/ml - Absorbance of L-Ascorbic acid	17.4035000	9.4287065	3.8492533	7.5086794	27.2983206	4.521	5	.006
Pair 5	Concentration µg/ml - %of Hydrogen peroxides scavenging activity of L-Ascorbic acid	-36.5928480	24.3700634	9.9490367	-62.1676611	-11.0180348	-3.678	5	.014

Pair 6	Absorbance of L-Ascorbic acid - %of Hydrogen peroxides scavenging activity of L-Ascorbic acid	-53.9963480	31.1342132	12.7104893	-86.6697009	-21.3229951	-4.248	5	.008
Pair 7	Concentration µg/ml - Absorbance of 2,4 Dinitro phenyl hydrzine	17.4710000	9.3617340	3.8219119	7.6464627	27.2955373	4.571	5	.006
Pair 8	Concentration µg/ml - %of Hydrogen peroxides scavenging activity of 2,4 Dinitro phenyly hydrazine	-67.1824552	9.3867973	3.8321439	-77.0332948	-57.3316156	-17.531	5	.000
Pair 9	Absorbance of 2,4 Dinitro phenyl hydrzine - %of Hydrogen peroxides scavenging activity of 2,4 Dinitro phenyly hydrazine	-84.6534552	.5210066	.2127000	-85.2002181	-84.1066923	-397.995	5	.000
Pair 10	Concentration µg/ml - Absorbance of aniline	17.4728333	9.3610391	3.8216282	7.6490253	27.2966414	4.572	5	.006

Pair 11	Concentration µg/ml - %of Hydrogen peroxides scavenging activity of aniline	-68.1190871	9.5582701	3.9021474	-78.1498763	-58.0882978	-17.457	5	.000
Pair 12	Absorbance of aniline - %of Hydrogen peroxides scavenging activity of aniline	-85.5919204	.4929994	.2012662	-86.1092916	-85.0745493	-425.267	5	.000
Pair 13	Concentration µg/ml - Absorbance of 2,4 DinitroAniline	17.4683333	9.3610924	3.8216500	7.6444693	27.2921974	4.571	5	.006
Pair 14	Concentration µg/ml - %of Hydrogen peroxides scavenging activity of 2,4 Dinitro Aniline	-65.5741735	10.2154600	4.1704441	-76.2946413	-54.8537056	-15.724	5	.000
Pair 15	Absorbance of 2,4 DinitroAniline - %of Hydrogen peroxides scavenging activity of 2,4 Dinitro Aniline	-83.0425068	.9961106	.4066604	-84.0878607	-81.9971529	-204.206	5	.000

IBM SPSS statistics 19 calculates this p-value to be less than 0.05.Given this *G.edulis* methanol extract and all tested compounds p-values are less than alpha of 0.05, first one rejected null hypothesis that concentration and their absorbance are independent. Second one rejected null hypothesis which concentration and hydrogen peroxide scavenging activity are

independent. Third one reject null hypothesis that Absorbance and its hydrogen peroxide scavenging activity are independent. We conclude that they are dependent, that there is an association between the two variables of all pairs. That means first variable concentration helps to predict the level of variable of absorbance. Second one Variable of concentration helps to predict the level of hydrogen peroxide scavenging activity. Then Variable of absorbance helps to predict the hydrogen peroxide scavenging activity.

The presence of antioxidants was measured by hydrogen peroxide scavenging activity. Spectroscopic absorbance measurements of the tested compounds were used to analyses the antioxidant activity. (Picture: 1) In this H_2O_2 scavenging activity of the second mechanisms, the tested compounds were easily lose their electrons that were been high scavenging activity and good antioxidants. That compounds were basic nature. L-ascorbic acid was higher absorbance followed by 2, 4-Dinitro aniline 2, 4-Dinitro phenyl hydrazine, Aniline and *G.edulis* methanol extract.(Fig1). In the figure-2, different concentration methanolic extract of *G.edulis* with H_2O_2 reduction, Standard L-ascorbic acid was used as the standard and increse from 2.459016 to 69.53125%. That was directly proportional to the concentration of the standard. *G.edulis* methanol extract was recorded higher H_2O_2 scavenging activity followed by Aniline, 2, 4-Dinitro phenyl hydrazine, 2, 4-Dinitro aniline and L-ascorbic acid. (Fig: 2). Concentration, absorbance and % hydrogen peroxide scavenging activity of all tested compounds variable were not independent. That meant those variable were depend on each other. ($P<0.05$).

DISCUSSION

The antioxidant activity of methanol extract of *G.edulis* was measured by H_2O_2 scavenging assay, the methanolic extracts of the *G.edulis* samples were tested in different concentrations from 5-30µg. In 5ug/ml methanol extract of *G.edulis* was shown higher H_2O_2 scavenging activity that means H_2O_2 reduction up to 89.95902% from 82.1875%. In 30ug/ml methanol extract of *G.edulis* was shown least H_2O_2 scavenging activity. After that it was been constant or steady state due to the constant amount of H_2O_2. The presence of antioxidant activity was shown in the results of H_2O_2 reduction method which was represented in table2. The comparative study 5ug/ml of methanol extract of *G.edulis* was recorded highest antioxidant activity and 30ug/ml was least antioxidant activity. But in 15ug/ml had shown steady state of antioxidant activity. While concentration of the methanol extract of *G.edulis* sample increase, H_2O_2 reduction or H_2O_2 scavenging activity was decreased. That meant methanol extract of *G.edulis* concentration inversely proportional to H_2O_2 reduction. .

However antioxidant property or inhibition rate had been correlated to their concentration of methanol extract of *G.edulis*. That meant these two were significant. Where illustrate with efficient electron donation reactions by antioxidant compounds and in turn produce phenoxyl radical species as intermediates of oxidizing agents. The inhibition percentage was varied depends on the concentration of the methanol extract of *G.edulis* used. The antioxidant activity of *G.edulis* was shown good peak than other tested aromatic compounds in figure2. In above all results, antioxidant potential of *G.edulis* was taken as accurate finding which was used for further intervention the effect of different processing condition on antioxidant activity of *G.edulis*.

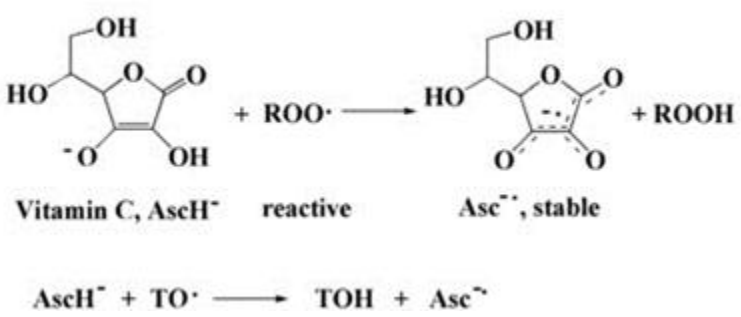


Figure: 3. Direct reactions of vitamin C ($AscH^-$) with ROO▪ (B)

Vitamin C contain L-ascorbic acid which is good antioxidants it has a property to remove its hydrogen atom by H_2O_2 free radical. Normally free radical need to be degenerate from the body. Otherwise it causes the diseases. If compound was removed its hydrogen easily that shown highest absorbance and lowest hydrogen peroxide scavenging activity. (Wright et al., 2001).

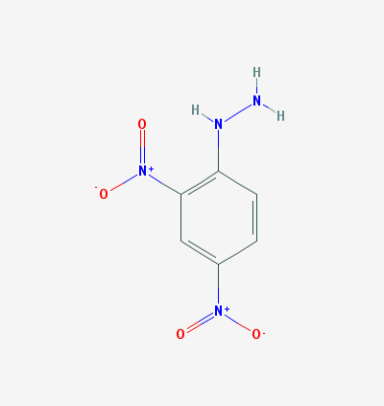


Figure:4 Molecular structure of DNPH

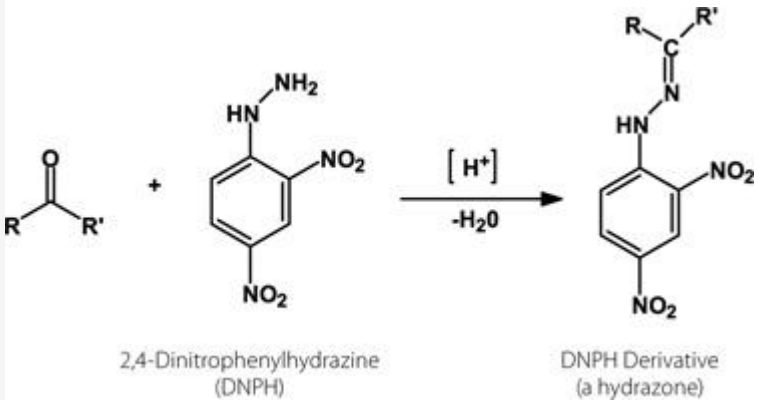


Figure:5 Reaction of Keton with 2,4-Dinitrophenylhydrazine

DNPH (2, 4-Dinitrophenylhydrazine) is called as Borche's reagent or Brady's reagent. DNPH is a red to orange powder. Which chemical compound is C_6H_3 $(NO_2)_2NHNH_2$ and its Molar mass 198.14 g/mol. It is a substituted hydrazine, and is often used to qualitatively test for carbonyl groups associated with aldehydes and ketones. Ketone react with 2, 4-Dinitrophenyl hydrazine show in above picture:4. It has an ability to donate electron by its NH_2 donor group for H_2O_2 reduction. This compound can give an electron to the free radical becoming itself a radical cation. But their ability is lower than aniline and *G.edulis* methanol extract and higher than 2, 4-Dinitro aniline and L-ascorbic acid.

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 04 Issue 09
August 2017

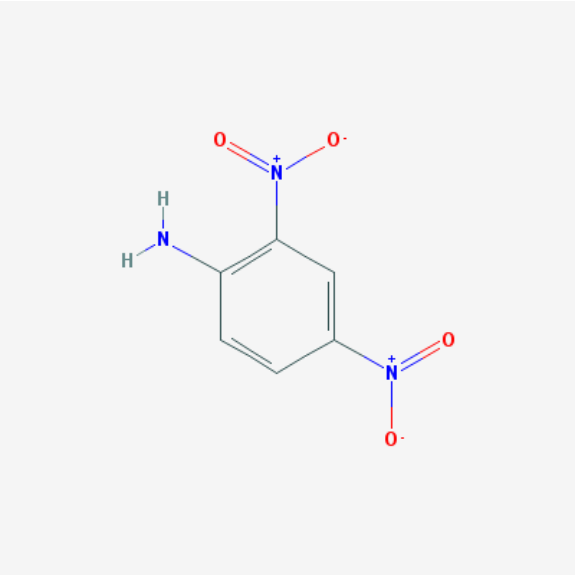


Figure:6 2,4-Dinitroaniline molecular structure

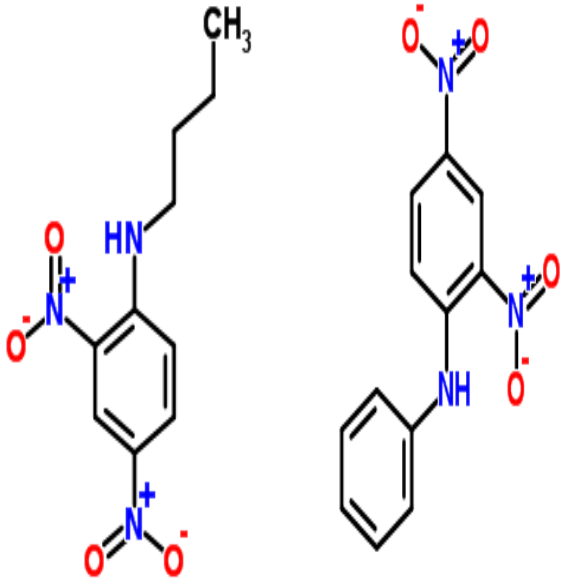


Figure:7 Reactions of 2,4-Dinitroaniline

2, 4-Dinitroaniline molecular weight is 183.123g/mol and its molecular formula is or $C_6H_3(NH_2)(NO_2)_2$. Two nitro electron withdrawing groups attach to benzene. Therefore it shows as acidic nature, low Electron donating ability but high hydrogen releasing ability.(Fig:7) Here absorbance by spectrophotometer (Dr 5000) was lower than L-absorbic acid and higher than methanol extract of *G.edulis,* Aniline and 2,4-Dinitrophenyl hydrazine. Therefore percentage of hydrogen peroxide scavenging activity lower than methanol extract of *G.edulis*, Aniline and 2, 4-Dinitrophenyl hydrazine but higher than L-absorbic acid.

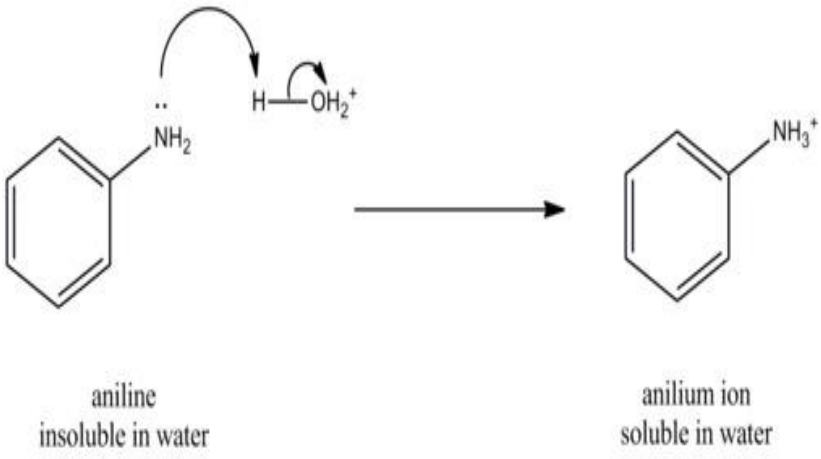


Figure: 8 Aniline molecular structure **Figure: 9** reactions of Aniline with other compounds

Aniline is the prototypical organic aromatic amine. Aniline Molar mass is 93.13 g/mol and its formula $C_6H_5NH_2$. Which is amine substituted one so it has strong antioxidant activity. (Iwatsuki et al., 1995). Aniline H_2O_2 scavennging activity described according to the

second mechanisms radical cation forming explained by Wright et al., 2001. Benzene with amine group called aniline which has the easiest electron donating ability (Fig: 9) due to its lower IP value. Hence H_2O_2-scavenging activity or antioxidant activity decreases due to NH_2 electron donor substitution groups gave a negative effect on IP value. So Antioxidant activity was higher than 2, 4-Dinitrophenyl hydrazine and 2, 4-Dinitroaniline and L-absorbic acid, but lower than methanol extract of *G.edulis.*

The H_2O_2 scavenging activity and its mechanisms illustrated in followed mechanisms $H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$ by Wethsinghe & shahah in 1999. Active groups that mean electron donating groups or electron withdrawing groups was largely influence on H_2O_2 scavenging activity and antioxidant activity. Aromatic benzene ring hydrogen atom substituted by electron donor groups such as NH_2 was increased H_2O_2 scavenging activity and known as good antioxidant that was clearly stated in the second mechanisms of H_2O_2 scavenging activity. Electron withdrawing group means it absorbs the electron was negatively effect on antioxidant activity. The presences of electron donating or withdrawing active groups in ortho position were more active followed by Para and Meta. Oxygen atom is high electronegativity than nitrogen atom that means nitrogen atom is good electron donating. Therefore aniline was showed high antioxidant activity and taken place 2nd order. In the 3rd order 2,4 dinitro phenyl hydrazine was recorded high antioxidant activity due to two nitrogen atom has free electrons but lower than aniline due to their electron withdrawing substitution. In the 4th order 2,4 Dinitro aniline was shown antioxidant activity but lower than aniline due to presence of electron withdrawing groups in ortho and para position. Active groups or substitution presence in ortho and para position in benzene ring was more active than meta position. (Ma et al., 2011). *G.edulis* methanol extract was recorded lowest absorbance than other tested compounds in spectrophotometer that meant it was shown highest H_2O_2 scavenging activity and act as good antioxidants than other tested compounds here due to its flavanol content. *G.edulis* methanol extract was taken place in 1st order for the antioxidant activity. H_2O_2 absorb electron from antioxidant compounds to accelerate antioxidant reaction where H_2O_2 absorbance by the spectrophotometer decrease owing to H_2O_2 changed into H_2O. Second mechanisms elucidated H_2O_2 scavenging activity where tested compounds transfer its electron easily that had high H_2O_2 scavenging activity and good antioxidants. On the other hand based on 1st mechanism shows the tested compound loose its hydrogen atom easily will goes to less H_2O_2 scavenging activity. Which aromatic rings has high resonance effect. Hence it has lower absorbance and highest antioxidant activity rather than other aliphatic compounds.

G.edulis is good natural source for bioactive secondary metaboilites separation. Which species can be work as natural antioxidants (Layse et al., 2011). Several research persons who were carryout to show its anti properties of *G.edulis*.(Divya et al.,2013). Under the Rhodophyta *G.edulis* contain Pigments which can absorb the rays in deep water situation for produce food molecules. This particular seaweed species is exposed to a combination of oxygen concentration in the harsh environment situation which can be lead to produce free radicals and strong oxidizing agents owing to that they are suffered serious photodynamic damage during its metabolism. This is the reason behinds seaweeds cells contain special compounds and have special mechanisms. (Divya et al.,2013).

Conclusion

In H_2O_2 scavenging activity assay *G.edulis* methanol extract was recorded highest antioxidant activity. Aniline was resulted 2nd highest H_2O_2 scavenging activity compare with all tested compounds here due to presence of electron donor amino group. Hence which group

has lower IP value and increase antioxidant activity. 3rd, 4th and 5th place of antioxidant activity are 2, 4-dinitrophenyl hydrazine, 2, 4-Dinitroaniline and L-ascorbic acid, Electron donor ability depend on H_2O_2 scavenging activity was explained as 2nd mechanism. (Wright et al., 2001). Such NH_2 electron donating substitution in aromatic ring gives positive effect. Electron withdrawing groups such NO_2 give negative effect to H_2O_2 scavenging activity. The presence of active groups was more active in ortho position followed by Para and Meta position. The absorbance of H_2O_2 was decreased; H_2O_2 scavenging activity was increased so the antioxidant activity was also increased to all tested compounds and *G.edulis* methanol extract. *G.edulis* was a remarkable antioxidant activity while compare with common aromatic tested compounds.While consider seaweeds resources, Sri Lanka is having rich bio diversity in the coastal marine environment. This *G.edulis species* under red algae group is widely distributed in the Northwest coast of Sri Lanka especially in Kalpitiya, Puttalam. The present research findings revealed that *G.edulis* is adequeatly exhibited successful antioxidant activities. Where Flavanoid, alkaloid, total phenol and saponins of *G.edulis* were been the reason to record highest antioxidant activity compare with other common laboratory tested aromatic compounds. And also these bioactive compounds of *G.edulis* are free radical scavengers and good potent natural phenolic antioxidants for commercial purposes around the world. Especially muslims people around coastal area of Sri Lanka eats Kanchi passi(*G.edulis*) has available rich antioxidant content . So this is promising research finding as there may be a good potential to use such *G.edulis* methanol extracts for protect the human from the degenerative diseases. *G.edulis* especially from North west Coast of Sri Lanka possess significant antioxidant potential and hence deserve that species in Biotechnology industry programs to develop a functional food to make the man in healthier life.

Reference

1. Akoh, C. C. and Min, B. D. (1997). Food Lipid Chemistry. In: Nutrition Biotechnology Marcel Dekker Inc., New York.

2. Al-Azzawie, H. F. and Mohamed-Saiel, S. A. (2006). Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Science*, **78,** 1371-1377.

3. Amin Ismail & Tan Siew HongAntioxidant activity of selectedcommercial seaweeds, *Mal J Nutr* **8(2)** 2002,pp.167 -177.

4. Aruoma, I. O. (1999). Antioxidant action of plant foods. Use of oxidative DNA damage, as a tool for studying antioxidant efficacy. *Free Radical Research,* **30**, 419-427.

5. Bansemir, A., Blume, M., Schroder, S. and Lindequist, U. (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, **252**, 79-84.

6. Bouhlal, R.-Haslin, C-Chermann, J.C.-Colliec-Jouault, S.-Sinquin, C-Simon, G. -Cerantola,S.-Riadi, H-Bourgougnon, N. (2011). Antiviral activities of sulfated Polysaccharides isolated from Sphaerococcuscoronopifolius (Rhodophyta, Gigartinales) andBoergeseniellathuyoides (Rhodophyta, Ceramiales). *In Marine Drugs*, vol. **9**: p. 1187-1209

7.Chandini et al., 2008S.K. Chandini, P. Ganesan, N. Bhaska, In vitro antioxidant activities of three selected brown seaweeds of India, *Food Chem.*, **107** (2008), pp. 707–71

8. Chanda S(2010), Seaweeds : A novel ,untapped source of drug from sea to combat infectious diseases, Current Research, Technology and Education Topics in Applied Microbiology & Microbial Biotechnology,**Vol 1**,pp.473-480.

9. Chandini, S. K., Ganesan, P. and Bhaskar N. (2008). In vitro antioxidant activities of three

selected brown seaweeds of India, *Food Chemistry*, **107**, 707-713.

10. Chew, Y. L., Lim, Y. Y., Omar, M. and Khoo, K. S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT*, **41**, 1067-1072.

11. Divya.S.R, Padmaja,M, Chandramohan,(2013) Analysis and comparisons of antioxidant activity *Gracilaria edulis* and *Gelidium.sp*, *International journal of Agricultural innovations and research*, **2**, 251-252

12. Ganesan, P., Kumar, C. S. and Bhaskar, N. (2008). Antioxidant properties of methanol extract and its solvent factions obtained from selected Indian red seaweeds. Bioresource Technology, **99**, 2717-2723.

13. Gonzalez del Val, A., Platas, G., Basilo, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo, E., Jimenez del Rico, M., Garcia Reina, G. and Pelez, F. (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Journal of Microbiology*, **4**, 35-40.

14. Haslam, E. (1989). Plant Polyphenols: Vegetable Tannins Revisited. Cambridge University Press, Cambridge, UK.

15. Harbourne, J. B. (1994). The Flavonoids: Advances in Research Since 1986. London, UK: Chapman and Hall.

16. Herrmann, K. (1989). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in food. *CRC Critical Reviews in Food Science and Nutrition*, **28**, 315-347.

17. Ito K, Hori K. Seaweed: Chemical composition and potential food uses. *Food Rev Int* **5**:101–144 (1989)

18. Jadhav, S.J. Nimbalkar, S.S. Kulkarni, A.D. & Madhavi, D.L. Lipid Oxidation In Biological and Food Systems. In: Food Antioxidants. Madhavi DL, Deshpande SS & Salunkhe DK (eds). New York. (1995)

19. Kasi Pandima Devi, Natarajan Suganthy, Periyanaina Kesika and Shanmugaiahthevar Karutha Pandian, "Bioprotective properties of seaweeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content", *BMC Complementary and Alternative Medicine*, **8**:38. (2008)

20. Lam, C. and Harder, T. (2007). Marine macroalgae effect abundance and community richness of bacterioplankton in close proximity. *Journal of Phycology*, **43**, 874-881.

21. Layse C F. de Almeida, Heloina de S. Falcao and Leonia M. Batista*2011. Bioactivities from Marine Algae of the Genus *Gracilaria. Int J Mol Sci*. **12(7):** 4550–4573.

22. Ma, X., Li, H., Dong, J., Qian, W., 2011. Determination of hydrogen peroxide scavenging activity of phenolic acids by employing gold nanoshells precursor composites as nanoprobes. *Food Chem*. **126**,
698–704.

23. Manach, C., Scalbert, A., Morand, C., Rémés, C. and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**, 727-747

24. Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masak, K., Masuda, Y., Takeuchi, T., Chihara, M., Yamamoto, Y., Niki, E. and Karube, I. (1997). A comparison of screening methods for antioxidant activity in seaweeds. *Journal of Applied Phycology*, **9**, 29-35.

25. Meenakshi,S D. Manicka Gnanambigai total flavonoid and invitro antioxidant activity of two seaweeds of Rameshwarem coast. Global journal of pharmacology **3(2)** 2009,pp 59-62

26. Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A., and Kanter, M. (2000) Cereal Foods World **45(2),** 59-63.

27. Neol Vinay Thomas and Se-Kwon kim, Health beneficial aspects of phloroglucinol derivatives from marine brown algae, Handbook of Marine Macroalgae: Biotechnology and Applied Phycology,2012.pp.378-386.

28. Pai-An Hwang, Chwen-Herng Wu, Shu-Yun Gau, Shih-Yung Chien and Deng-Fwu Hwang, "Antioxidant and immune-stimulating activities of hot-water extract from seaweed *Sargassum hemiphyllum", Journal of Marine Science and Technology,* **18(1)**: pp. 41-46 (2010)

29. 4. Pisani, P. Bray, F. Parkin, D.M. Estimates of the world-wide prevalence of cancer for 25 sites in the
adult population. *Int J Cancer* **97**:71–81.

30. Plaza, M., Santoyo, S. Jaime, L., García-Blairsy Reina, G., Herrero, M., Senoráns, F.J. and Ibánez, E. (2009). Screening for bioactive compounds from algae. *Journal of Pharmaceutical and Biomedical Analysis*, **51 (2),** 450-455.

31. Ragan, M. A. and Glombitza, K. W. (1986). Phlorotannins, brown algal polyphenols. In: Hellebustad, JA, Craigie, JS (Eds), Handbook of Phycological Methods, Vol II. Cambridge University Press, Cambridge, (pp. 129-241).

32. Reaven, P. D. and Witzum, J. L. (1996). Oxidised LDL in atherogenesis. Role of dietary modification. *Annual Review of Nutrition*, **16**, 51-71.

33. Russo, N., Toscano, M., Uccella, N., 2000. Semiempirical molecular modeling into quercetin reactive site: structural, conformation, and electronic features. *J. Agric. Food Chem.* **48 (8),** 3232–3237.

34.Shukla, V. K., Wanasundara, P. K. and F. Shahidi. 1997. Natural antioxidants from oilseeds. In: (Shahidi, F. eds), *Natural antioxidants: Chemistry, health effects, and applications*. AOCS Press, Champaign, IL. pp. 97-132.

35. Skibola, C., The effect of Fucus vesiculosus, an edible brown seaweed, upon menstrual cycle length and hormonal status in three pre-menopausal women: a case report. *BMC Complementary and Alternative Medicine* **4**, 10–17 (2004).

36. Umakanthan G, Vinobaba P, Radampola K.,(2016)Phytochemical constitution of cultured *G.edulis* from the east coast of Batticaloa District, in Sri Lanka. 1st International conference at Trinco campus, Eastern University, Sri Lanka Konesapuri, Nilaveli Sri Lanka.

37. Varahalarao vadlapudi and K. Chandrashekar Naidu, In vitrobioevaluationofantioxidant activities of selected marine algae, *Journal Of Pharmacy Research* **3(2),**2010,pp.329-331

38. Wallace, G. and Fry, S. C. (1994). Phenolic components of the plant cell wall. *International Review of Cytology*, **151**, 229-267.

39.Wang, B.G, Zhang, W.W, Duan, X.J, Li, X.M.2009. Food Chem.,113:1101,1105,*Pharmacogn*,1997, **35**, 237-243.9.

40. Waterman, P. G. and Mole, S. (1994). Analysis of Phenolic Plant Metabolites. In: *Methods in Ecology*. Blackwell Scientific Publications, Oxford, UK.

41. Wettasinghe, M., Shahidi, F., 1999. Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *J. Agric. Food Chem*. **49**, 1801–1812.

42. Wright, J.S., Johnson, E.R., Di Labio, G.A., 2001. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J. Am. Chem. Soc.* **123 (6),** 1173–1183.

43. Yamaguchi, T. Takamura, H. Matoba, T. & Terao, J. HPLC method for evaluation of the free radicalscavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem* **62**:1201-1204 (1998)

44. Yuan, Y. V., Carrington, M. F. and Walsh, N. A. (2005). Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation *in vitro. Food and Chemical Toxicology*, **43**, 1073-1081.