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ESTIMATION OF TOTAL PHENOLIC, FLAVONOID AND ANTIOXIDANT OF METHANOLIC EXTRACT OF *THALASSIOSIRA WEISSFLOGII*

Ashashalini[1], M. Syed Ali*[1], V. Anuradha[2], V. Suganya[2] and P. Bhuvana[2]

[1*]PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, Tamil Nadu, India.

[2]PG & Research Department of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, Tamil Nadu, India.

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***Corresponding Author**
Dr. Syed Ali M.
PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, Tamil Nadu, India.

ABSTRACT

Marine algae contain a wide assortment of bioactive compounds, which have viable applications in pharmaceutical, medical, cosmetic, nutraceutical, nourishment and agricultural fields. Natural antioxidants, found in Diatoms show the vital role against numerous diseases and ageing progressions through assurance of cells from oxidative damage. The *in vitro* antioxidant assay, total phenolic content and total flavonoid content of methanolic extract of *Thalassiosira weissflogii* were performed along with standard drug like ascorbic acid, BHT, Gallic acid etc., Diatom *Thalassiosira weissflogii* was extracted using methanol solvent. The maximum phenolic content and flavonoid content of test sample was 11.34 and 2.06 mg equivalent to standard drug. All other antioxidant activity like DPPH, Nitric oxide, SOD, SO, LPO and β carotene shows better result than the standard drug. Thus, the result suggested that methanolic extract diatom *Thalassiosira weissflogii*could lead their application in many useful health cares also in chemoprevention of a variety of diseases including cancer.

KEYWORDS: Total phenolic content, total flavonoid content, diatom *Thalassiosira weissflogii*, DPPH and reactive oxygen species.

INTRODUCTION

Oxidative stress is the consequence of an awkwardness between pro-oxidant and antioxidant homeostasis that prompts to the generation of toxic, oxidative damage caused due to reactive

oxygen species on lipids, proteins and nucleic acids may trigger different chronic diseases, for example coronary heart disease, atherosclerosis, cancer and ageing.[1]

Oxygen free radicals crumble DNA, destroy cell membranes, and make havoc among cell's basic enzymatic metabolic processes.[2] Among the major causative factors in initiation of numerous chronic and degenerative infections including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is complicated in aging.[3]

Free Reactive oxygen species (ROS) respond effectively with free radicals to develop radicals themselves. ROS are different types of initiated oxygen, which comprise free radicals for example, superoxide anion radicals (O_2) and hydroxyl radicals (OH), as well as non-free radical species (H_2O_2) and the singled oxygen (O_2). The antioxidant action of these mixes are the most part attributed to scavenging activity against superoxide and hydroxyl radicals, chelating ability, quenching singlet andtriplet oxygen, and reducing power.[4,5]

It is essential to create, recognize and use new source of safe and effective antioxidants of natural origin.[6,7] There are numerous synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), Propylgallate (PG) and butylatedhydroxyquinone (TBHQ) which are commercially available and used currently.

Though, the use of these synthetic antioxidants for nourishment or medicine constituents has been stricted by the toxicity and safety that can prompt to the complications of the potential health in human. Because of the reasons, several scientists have tried to discover the more active oxidation inhibitors that might be utilized as antioxidants for foodstuff or drug compositions without the symptoms for the past long years. Such huge numbers of researchers have focussed tomany types of natural antioxidants that can be utilized without harmfulness in human.

The microalgae are good wellsprings of natural antioxidants.[8,9] At the time of photosynthesis process they retain solar light which is altered into chemical energy, later utilized in the conversion of CO_2 into carbohydrates, lipids, and in the meantime, creating molecular oxygen, which can achieve locally high concentration levels.

As, oxygen is effortlessly stimulated by ultraviolet radiation (UV) or heat from sunlight into lethal reactive oxygen species (ROS), plants and microalgae have built up a defensive mechanism which contains in the preparation of antioxidant compounds that able to minimize

the concentration of these ROS.[10] Even though, macro algae have received more consideration as potential natural antioxidants.[11, 12] there has been very restricted evidence on antioxidant activity of microalgae.[13,14] Few studies reported that cancer was prevented by algal extracts[15] due to their antioxidant properties.[16]

Thalassiosira weissflogii is a phytoplankton which belongs to the essential group of prokaryotes. It is also called diatoms which contributing over 40% of total primary production in Ocean**.** Carbon production is the extreme source of diatoms, since diatom have siliceous carbon dioxide and light.[17] Diatom have good source of natural antioxidant. Generally, various methods are used to investigate antioxidant properties from, seaweed[18], micro algae[19], plant extract. In present study, the antioxidant activity of methanolic extract of *Thalassiosira weissflogii* has been confirmed by various methods.

MATERIALS AND METHODS

1. Sample preparation

1 gram dried powder of *Thalassiosira weissflogii* was mixed with 10 ml of methanol solvent and it is kept at room temperature for 24 hours. This mixture was filtered using Whatmann No.1 filter paper and stored at 4ºC for further analysis.

2. *In vitro* antioxidant activity

Total phenolic content

Total phenolic substances were assessed by following strategy: 100 µl of methanol extract of *Thalassiosira weissflogii* was blended with 2.0 ml of 2% Na_2CO_3 and permitted to remain for 2 min at room temperature. After incubation, 100 µl of 50% Folin Ciocalteau's phenol reagent was supplemented and was mixed thoroughly. It is then allowed to stand at room temperature for 30 min. Absorbance of all the samples were measured at 720 nm using spectrophotometer. Gallic acid was used as standard to determine total phenolic activity. Concentrations in the range of 100 to 500 µl were taken for each sample.[21]

Total flavonoid content

The total flavonoid content of tests was detected by the aluminum chloride colorimetric method.[22] 0.5 ml of methanol extract of *Thalassiosira weissflogii* were mixed with 250 µl of 5% sodium nitrite ($NaNO_2$) solution and 150 µl of 10% $AlCl_3$ solution and incubated for 5 mins. At that time, 0.5 ml of 1 mol/L sodium hydroxide (NaOH) solution was added, and was brought to 2.5 ml with double-distilled water. The mixture was allowed to stand for 15 min

which was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg. Rutin was used as standard (equivalent per g dry weight). The concentration in the range of 100 to 500 µl was taken.

Total antioxidant activity

The total antioxidant capacity of methanol extract of *Thalassiosira weissflogii* was evaluated by.[23] At first, 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2356 g of ammonium molybdate (4mM solution) was dissolved in distilled water and made up to 250 ml which was marked as TAC reagent. Then, to 300 µl of test sample 3 ml of TAC reagent was added. Reaction mixture was incubated at 95° C for 90 minutes. Absorbance was measured at 695 nm and ascorbic acid was used as standard. The concentration in the range of 100 to 500 µl were taken for both standard and test samples.

DPPH radical scavenging assay

The scavenging activity of methanol extract of *Thalassiosira weissflogii* for DPPH radical were identified by the method.[24] Concisely, 2.0 ml of test samples and 2.0 ml of 0.16 mM DPPH methanolic solution was mixed together. The mixture was vortexed for 1 min and then allowed to stand at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The varied concentration in the range of 100 to 500 µl was taken for both standard and methanol extract of *Thalassiosira weissflogii*. The scavenging effect (%) was calculated by using the formulae.

Scavenging effect (%) = (Absorbance of control –Absorbance of test solution)/Absorbance of control] × 100

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was done based on the procedure.[25] A solution of hydrogen peroxide (H_2O_2, 10 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). 3.4 ml of phosphate buffer was mixed with 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM) and 1ml (0.25 mg) of methanol extract of *Thalassiosira weissflogii* was added to it. The absorbance value of the mixture was recorded at 230 nm after 10 minutes and incubation at room temperature. Blank solution contains sodium phosphate buffer without H_2O_2 was used. Ascorbic acid was used as the standard. The percentage of H_2O_2 scavenging of crude extract and standard compounds were calculated using the following equation.

H_2O_2 scavenging effect (%) = (Absorbance of control –Absorbance of test solution)/Absorbance of control) × 100

Nitric oxide scavenging activity

Nitric oxide scavenging activity was performed based on the following procedure: 3ml of 10 mM of sodium nitroprusside was prepared in phosphate buffer saline (pH 7.4, 0.2 M) which was mixed with 1 ml of methanol extract of *Thalassiosira weissflogii* and incubated at 25°C for 180 mins. By mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid the Griess reagent was prepared immediately before use. The sample was mixed with an equal volume of freshly prepared Griess reagent. The absorbance was measured at 546 nm. Ascorbic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded.[26]

The percentage nitrite radical scavenging activity of the ethanol extracts and Gallic acid were calculated using the following formula.

Nitric oxide activity (%) = Absorbance of control- Absorbance of test / Absorbance of control × 100.

Ferric reducing antioxidant Power (FRAP)

Reducing power of methanol extract of *Thalassiosira weissflogii* was carried out by the method prescribed by.[27] To 1.0 mL of methanol extract of *Thalassiosira weissflogii*, 2.5 ml of Phosphate buffer (0.2 M, pH 6.6) and 2.5 mL Potassium ferricyanide (1%) was mixed. Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of Trichloroacetic acid (10%) was added and centrifuged (650 g) for 10 min. From the upper layer, 2.5 mL solution was taken and mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Increased absorbance is indicated increased reducing power.

Deoxyribose Radical Scavenging Activity

Deoxyribose non-site specific hydroxyl radical scavenging activity of methanol extract of *Thalassiosira weissflogii* was estimated.[28] Briefly, 2.0 ml aliquots of test samples were mixed to the test tube containing reaction mixture of 2.0 ml $FeSO_4.7H_2O$ (10mM), 0.2 ml EDTA (10mM) and 0.2 ml deoxyribose (10mM). The volume was made up to 1.8 ml with phosphate buffer (0.1M, pH-7.4) and to that 0.2 ml H_2O_2 (10mM) was added. The mixture was incubated at 37°C under dark for 4 hours. After incubation, 1 ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then kept under boiling water bath for 10 min. After the treatment the samples were absorbed at 532nm. If the mixture was turbid, the

absorbance was measured after filtration. Ascorbic acid was used as standard. Scavenging activity (%) was calculated using the equation.

Deoxyribose radical scavenging activity (%) = Absorbance of control- Absorbance of test / Absorbance of control × 100

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Assay

Free radical scavenging activity was determined by ABTS radical cation decolourization assay.[29] ABTS radical cation was creäted by mixing 20mM ABTS solution with 70mM potassium peroxodisulphate and kept to stand in dark at room temperature for 24 hours before use. To, 0.6 ml of methanol extract of *Thalassiosira weissflogii* (0.25 mg), 0.45 ml of ABTS reagent was added and absorbance of these solutions was measured at 734 nm after 10 min.

ABTS radical cation scavenging assay [%] = Absorbance of control- Absorbance of test / Absorbance of control × 100

Superoxide radical scavenging activity

Scavenging of superoxide radical was calculated using the method elaborated by.[30] Assay tubes contained 0.2 ml of methanol extract of *Thalassiosira weissflogii* (corresponding to 20 mg extract) with 0.2 ml EDTA (12mM), 0.1 ml Nitro blue tetrazolium, 0.05 ml riboflavin (20µg) and 2.64 ml phosphate buffer (50 mM, 7.6 pH). The control tubes were set up with DMSO (Dimethyl sulfoxide) solution instead of the test solution. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 mins. A560 was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition was calculated by comparing with O.D of the control tubes.

SOD activity

Measurement of superoxide anion scavenging activity was performed based on the method[31], with slight modifications. To, 1ml of Nitro blue Tetrazolium (NBT) solution containing 156µM NBT dissolved in 1.0 ml of phosphate buffer (100mM, pH 7.4) and 1ml of NADH solution containing 468 µM of NADH which is dissolved in 1ml of phosphate buffer (100 mM, pH 7.4) with 0.1 ml of test samples were added and the reaction was started by adding 100 µl of Phenazine methosulphate (PMS) solution containing 60 µM of PMS 100 µl of phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560nm was measured against the control samples. BHT was used as the

reference compounds (200 to 1000 µg/ ml). The percentage of inhibition was calculated as mentioned below.

% of SOD = (Absorbance of control- Absorbance of test) / Absorbance of control × 100

Estimation of lipid peroxidation using egg yolks

Inhibitions of lipid peroxidation in the egg of hen were determined using a modified method thiobarbituric acid- reactive species (TBARS) assay.[32] Egg homogenate (0.5 ml, 10% in distilled water, v/v) was mixed with 0.1 ml of methanol extract of *Thalassiosira weissflogii* in a test tube and the volume was made up to 1 ml, by adding distilled water. Lastly, 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture to induce lipid peroxidation and incubated for 30 min. Then, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA (w/v) in 1.1% sodium dodecyl sulfate (SDS) and 0.05 ml 20% TCA was added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm.

% Inhibition= Absorbance of control- Absorbance of test / Absorbance of control × 100

β carotene linoleic acid assay

β- Carotene linoleic acid assay was carried out based on.[33] Briefly, in 10 ml of chloroform, 2 mg β-carotene, 200 mg linoleic acid and 20 mg Tween 40 were dissolved which was taken in flask. Chloroform was evaporated using vacuum evaporator apparatus. At that time, 50 ml of distilled water saturated with oxygen by shaking for 30 mins. This mixture is used as stock solution. 200 µl of methanol extract of *Thalassiosira weissflogii* were mixed with 2.5 ml of stock solution in the test tube. After that, the samples were placed in an oven at 50°C for 3 hours. The absorbance was read at 470 nm.

The percent of antioxidant activity was calculated from the following equation:

% Inhibition= Absorbance of control- Absorbance of test / Absorbance of control × 100

RESULTS AND DISCUSSION

Microalgae represent a relatively unexploited resource of natural antioxidants, because of their huge biodiversity, significantly more varied than higher plants. The marine diatoms are contributing around 80% of total phytoplankton biomass. Antioxidants are playing main role on the inhibition of lipid peroxidation for food protection and prevention of living cells against oxidative destruction. Total phenolic content and total Flavonoid content of methanolic extract of diatom *Thalassiosira weissflogii* were determined along with standard

Gallic acid and the results were presented in **Figure 1** and **2**. Total phenolic content was found to be 11.34 mg equivalent Gallic acid/ g dw and total flavonoid content was found to be 2.06 mg equivalent Gallic acid/ g dw (**Table 1**).

Total antioxidant activity of methanol extract of *Thalassiosira weissflogii* were presented in (**Table 2**). It is founded that the OD of Test sample ranges from 0.006 ±0.013 to 0.021 ± 0.0111 and standard ascorbic acid ranges from (0.002 ± 0.006 and 0.017 ± 0.011).

The DPPH scavenging activity was done for methanol extract of *Thalassiosira weissflogii* along with standard ascorbic acid (**Table 3**). For the standard, highest inhibition of 64.71% was shown at the concentration 500 µg/mland the lowest inhibition was found at the concentration 100 µg/ml (13.72%) At all concentration test samples possesses higherpercentage of inhibition (19.58% to 72.92%) when compared withstandard drug. The IC 50 value of both standard and methanol extract was founded to be 403.815 and 363.723 µg/ml.

In the present study, hydrogen peroxide scavenging assay of methanol extract of *Thalassiosira weissflogii* was determined and it was compared with the standard ascorbic acid which is shown in **Table 4**. The scavenging capacity of *Thalassiosira weissflogii* methanol extract was found to be 88.09% at 500 µg/ withthe IC50 value 242.524µg/ml and standard exhibit 84.26% at 500 µg/ withthe IC50 value 267.800 µg/ml.

Percentage of inhibition of nitric oxide scavenging assay of methanol extract of *Thalassiosira weissflogii* along with standard was done and recorded in **Table 5**. 14.53% at 100 µg/ml and 78.75% at 500 µg/ml concentration was obtained for methanol extract of *Thalassiosira weissflogii* and 15.66% at 100 µg/ml and 76.33% at 500 µg/ml concentration was gained by standard drug. Therefore, the percentage of inhibition by test sample is higher than the standard drug**.** The IC 50 value of standard was 302. 609 µg/ml and the test sample was 339.527 µg/ml.

The reducing power capacity of methanol extract of *Thalassiosira weissflogii* compared with standard drug. The OD value of the standard in the range of 0.012 to 0.062 was recorded at concentration 100 – 500 µg/ml which shows lower activity than methanol extract that possess OD range of 0.019 to 0.051. The values were represented in **Table 6.**

For the methanolic extract of *Thalassiosira weissflogii* Deoxyribose radical scavenging assay was carried out which produces 73.94% and the standard produces 82.45% at the concentration 500 µg/ml. It is founded that test sample exhibit lower activity than the standard drug. Hence, the IC50 value of methanol extract of *Thalassiosira weissflogii* was 262.085µg/ml (**Table 7**).

Table 8 indicates the percentage of inhibition against concentration in the range of 100 – 500 µg/ml of both drug standard and test samples. The methanol extract of showed maximum of 81.25% of inhibition at 500 µg/ml concentrations with IC 50 values 249.226 µg/ml and they are slightly higher than that of the standard drug (78.66%) with IC 50 value 260.203 µg/ml.

Superoxide radical scavenging activity of *Thalassiosira weissflogii* and the standard drug was assessed and recorded along with IC 50 values (**Table 9**). Decreasing in OD values tends to increase the percentage of inhibition. The superoxide radical scavenging activity of Standard drug (88.70%) was lower than the test sample (90.91%) at 500 µg/ml with IC 50 values of 230.790 µg/ml (standard drug) and 216.697 µg/ml (Test sample).

Table 10 explain the superoxide dismutase scavenging activity of standard (17.01% at 100 µg/ml and 82.41% at 500 µg/ml) and *Thalassiosira weissflogii* (34.58% at 100 µg/ml and 84.44% at 500 µg/ml) with IC 50 value 230.920 µg/ml (test sample) and for standard IC 50 value is 300.957 µg/ml.

Estimation of lipid peroxidation using egg yolks was carried out for standard and methanol extract of *Thalassiosira weissflogii***. Table 11** explains the LPO scavenging activity by egg yolk of standard and *Thalassiosira weissflogii*which shows 19.07% at 100 µg/ml and 79.70% at 500 µg/ml with IC 50 value 323.368 µg/ml for test samples and standardexhibit 17.70% at 100 µg/ml and 78.01% at 500 µg/ml which is slightly higher than the standard drug and possess IC 50 values of 313.030µg/ml.

The β caroten linoleic activity of *Thalassiosira weissflogii* along with standard the concentration in the range of 100 – 500µg/ml was performed (**Table12**). The minimum activity was founded for methanol extract (22.10%) and the standard drug (18.48%) at the concentration 100 µg/ml. At concentration 500 µg/ml methanol extract (87.39%) showed higher activity than standard (83.74%). The IC 50 values of both standard and test samples

were calculated which was found to be 266.632µg/ml and 258.380 µg/ml based on their percentage of inhibition.

Microalgae have described to contain carotenoids, vitamin E, phycocyanin, and chlorophyll.[34] These compounds are well known to decrease DPPH radicals by their hydrogen-donating ability.[35-37] The metal-chelating action is similarly used in assessing the antioxidant activity of various natural products.

The phenolic compounds (phenolcarboxylic acids and their derivatives, catechols, flavonoids and carotenoids etc.) are thought to be foremost contributors of antioxidant capacity in microalgae.[38] These antioxidants also have different biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities.

In present evaluation, the complete result demonstrated that the better antioxidant activity was established by methanolic extract of *Thalassiosira weissflogii* when compared with other standard like ascorbic acid, BHT, Gallic acid etc., (Concentration 500 µg/ml). Consequently, the outcome was compared with others findings with various marine diatoms.

In the previous research[39], the correlation co-efficient demonstrated that the phenolic contents are lesser cause of antioxidant activity because the solvent acetone was used in this study. Thus, it is assumed that the pigments are foremost component for the antioxidant, DPPH radical scavenging activity and FRAP. The microalgae have described that the presence of pigments like chlorophylls and carotenoids.

The investigation of[40,41], assessed the antioxidant properties and total phenolic content in three various solvent extract of three certain marine diatoms, *Chaetoceroscurvisetus*, *Thalassiosirasubtilis* and *Odantellaurita*. Results exhibited that total phenolic content and antioxidant properties were higher in methanolic extract of *O. aurita* while, DPPH radical scavenging activity (15.25%), hydrogen peroxide radical scavenging activity (54.73%), Ferric reducing power assay (1.032 ± 0.031 mg/g ascorbic acid equivalent) and nitric oxide radical scavenging were maximum in *C. curvisetus* (32.37%).

The present outcome was also compared with the earlier investigation[42] and it clearly explain that the methanolic extract was observed to have higher phenolic content in *D. Olivaceous* and flavonoid content was high in acetone extract of *C. humicola*. As indicated by[43] methanol extract of *Chlorella marina* exhibited higher action which was followed by

diethyl ether and hexane extract. This might be because of the differences in the polarity of the solvents used.

Likewise,[44] revealed that the highest antioxidant activity was detected in methanol extract from eight greenalgal species. In other study, it is founded that, the methanolic extracts exhibited greater potential in all antioxidant assays when compared to ethanolic and acetone extract of green microalgae *Desmococcus olivaceous* and *Chlorococcum humicola*.

In[41] research, methanol extract of *C. marina* (23.08%) and *D. Salina* (17.66%) and acetone extract of *C. Marina* (20.54%) was found to be the utmost potent scavenger. The hexane extract of *N. Clavata* exhibited the minimum DPPH radical scavenging activity at 9.1% respectively. Likewise, both methanolic and acetone extracts of *D. olivaceous* and *C. humicola* showed a significant dose dependent reduction of DPPH radicals,[42, 45] revealed that the 80% methanol extract and organic solvent fractions of both algae exposed prominent activities representing the higher efficacy for scavenging of free radicals. The suggestions are important as radical scavengersmay protect cell tissues from free radicals, thereby preventing diseases like cancer.

Hydrogen peroxide Radical Scavenging Activity (%) was observed to be maximum in methanol (61.33%) and hexane extract (55.3%) of *C. marina*, while minimum in acetone extract (15.54%) of *N. clavata*.[34] Based on the report of[35] methanolic extracts of *D. olivaceous* revealed 39% scavenging activity and the acetone extracts of *C.humicola* revealed 15% scavenging activity. In this study, highest nitric oxide scavenging activity was detected in methanol and hexane extract of *C. marina* (25.76% and 21.73%) respectively. This is fixed with the finding of[54], who found that the ethyl acetate of *H. porphyrae* (30.1%) and the 80% of methanol extract of *O. unicellularis* (49.3%) displayed ominously higher nitric oxide radical scavenging effects than those of the commercial antioxidants.

Hence, from the above research report it is considered that in these marine diatoms phenolic content alone doesn't play a major contributor to the antioxidant but also contain different components. Thus, the antioxidant in marine diatom helps to cure various diseases like cancer, diabetic etc.

TABLES AND FIGURES

Table 1: Total Phenolic Content and Total Flavonoid Content of methanol extract of *Thalassiosira weissflogii.*

Test name	mg equivalent Standard drug/ g dw
Total Phenolic Content	11.34 mg equivalent Gallic acid/ g dw
Total Flavonoid content	2.06 mg equivalent Gallic acid/ g dw

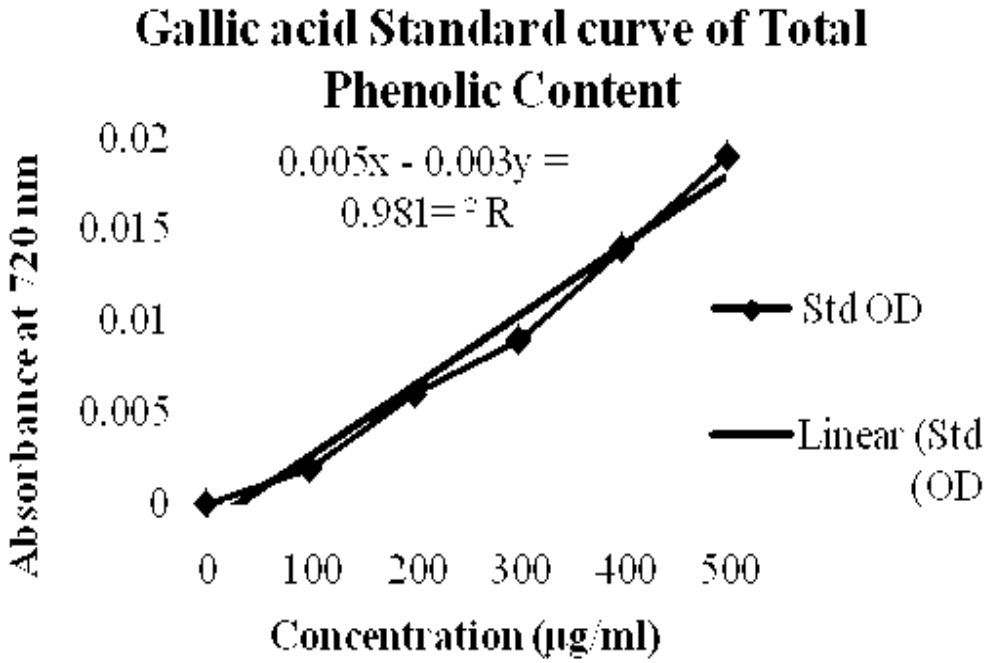

Figure 1: Total phenolic content of Gallic acid standard.

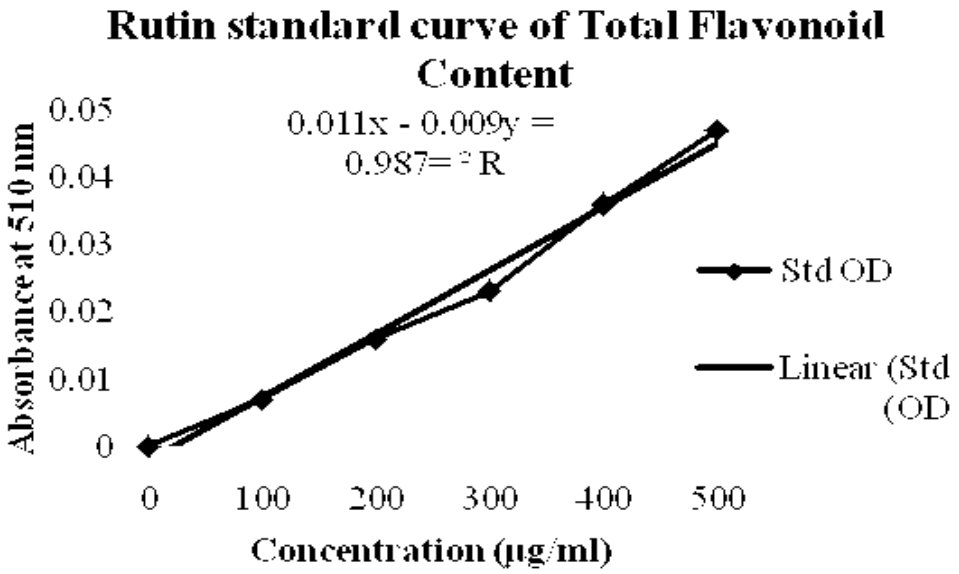

Figure 2: Total flavonoids content of Rutin standard.

Table 2: Total antioxidant activity of standard and methanol extract *In vitro* Antioxidant and Free Radical scavenging activity of methanol extract.

S.No	Concentration µg/ml	Standard OD Mean ± SD	Methanol extract OD Mean ± SD
1	**100**	0.002 ± 0.006	0.006± 0.013
2	**200**	0.006 ± 0.013	0.011± 0.022
3	**300**	0.008 ± 0.009	0.013± 0.028
4	**400**	0.012 ± 0.005	0.017± 0.043
5	**500**	0.017 ± 0.011	0.021 ± 0.111

Table 3: DPPH radical scavenging assay of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	**100**	13.72 ± 0.153	19.58 ± 0.033
2	**200**	25.90 ± 0.108	31.76 ± 0.012
3	**300**	38.17 ± 0.387	38.80 ± 0.254
4	**400**	44.77 ± 0.472	47.83 ± 0.002
5	**500**	64.71 ± 0.186	72.92 ± 0.045
IC 50 Values		403.815	363.723

Table 4: Hydrogen peroxide scavenging activity of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	**100**	23.68 ± 0.002	29.20 ± 0.001
2	**200**	40.50 ± 0.010	42.76 ± 0.066
3	**300**	54.91 ± 0.005	58.64 ± 0.256
4	**400**	71.08 ± 0.008	74.19 ± 0.058
5	**500**	84.26 ± 0.012	88.09 ± 0.011
IC 50 Values		267.800	242.524

Table 5: Nitric oxide scavenging activity of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	**100**	14.53 ± 0.006	15.66±0.19
2	**200**	33.25 ± 0.002	29.40±0.002
3	**300**	54.03 ± 0.120	45.24±0.254
4	**400**	67.32 ± 0.011	54.44±0.001
5	**500**	78.75 ± 0.008	76.33±0.022
IC 50 Values		302.609	339.527

Table 6: Ferric reducing antioxidant power of standard and methanol extract.

S.No	Concentration µg/ml	Standard OD Mean ± SD	Methanol extract OD Mean ± SD
1	100	0.012 ± 0.322	0.006 ± 0.005
2	200	0.026 ± 0.120	0.019 ± 0.011
3	300	0.039 ± 0.005	0.028± 0.001
4	400	0.051 ± 0.111	0.041 ± 0.022
5	500	0.062 ± 0.158	0.055 ± 0.122

Table 7: Deoxyribose Radical Scavenging Activity of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ±SD
1	100	23.00 ± 0.012	26.66 ± 0.002
2	200	36.52 ± 0.009	51.09±0.011
3	300	54.76 ± 0.002	55.21±0.236
4	400	69.10 ± 0.011	63.35±0.055
5	500	82.45 ± 0.003	73.94±0.011
IC 50 Values		279.099	262.085

Table 8: ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Assay of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	100	29.96 ± 0.018	31.10±0.001
2	200	40.90 ± 0.005	42.27±0.055
3	300	57.59 ± 0.010	57.45±1.025
4	400	67.58 ± 0.004	70.58±0.555
5	500	78.66 ± 0.122	81.25±0.001
IC 50 Values		260.203	249.226

Table 9: Superoxide radical scavenging activity of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	100	29.14 ± 0.007	30.72±0.002
2	200	42.62 ± 0.011	42.27±1.023
3	300	66.11 ± 0.003	69.44±0.003
4	400	76.31 ± 0.012	84.31±0.012
5	500	88.70 ± 0.011	90.91±0.365
IC 50 Values		230.790	216.697

Table 10: Superoxide Dismutase scavenging activity (SOD) of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	**100**	17.01 ± 0.003	34.58 ± 0.256
2	**200**	32.78 ± 0.001	41.72 ± 0.001
3	**300**	49.88 ± 0.121	60.25 ± 0.011
4	**400**	67.13 ± 0.101	74.92 ± 0.003
5	**500**	82.41 ± 0.004	84.44 ± 0.005
IC 50 Values		300.957	230.920

Table 11: Estimation of LPO (Egg yolks) for standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ± SD
1	**100**	17.70 ± 0.010	19.07±0.002
2	**200**	32.34 ± 0.004	30.80±0.066
3	**300**	45.76 ± 0.012	43.33±0.005
4	**400**	66.13 ± 0.006	59.57±0.011
5	**500**	78.01 ± 0.002	79.70±0.222
IC 50 Values		313.030	323.368

Table 12: β carotene linoleic acid assay of standard and methanol extract.

S.No	Concentration µg/ml	Standard % Mean ± SD	Methanol extract % Mean ±SD
1	**100**	18.48 ± 0.002	22.10±0.333
2	**200**	40.41 ± 0.021	41.62±0.002
3	**300**	60.10 ± 0.101	60.10±0.001
4	**400**	74.78 ± 0.011	72.36±0.005
5	**500**	83.74 ± 0.004	87.39±0.001
IC 50 Values		266.632	258.380

CONCLUSION

The outcome obviously demonstrated that the diatom *Thalassiosira weissflogii* exhibited higher antioxidant activity than the synthetic standard drug. However, based on our understanding, this was the primary report of examination on antioxidant properties of methanolic extract of *Thalassiosira weissflogii.* For that reason, advance examination is required to test the anticancer activity against Hepatocellular carcinoma by *in vitro* and *in vivo* animal model.

REFERENCES

1. Finkel T and Holbroo NJ. Oxidants, oxidative stress and the biology of ageing. Nature, 2000; 408: 239–247.
2. Kerr ME et al. An introduction to oxygen free radicals. Heart and Lung, 1991; 25: 200-209.
3. Halliwell B. The antioxidant paradox. Lancet, 2000; 355: 1179-1180.
4. Ruberto G et al. Antioxidant activity of extracts of the marine algal genus Cystoseira in a micellar model system. J. Appli. Phycol, 2001; 13: 403-407.
5. Athukorala Y et al. Anti- proliferative and antioxidant properties of an enzymatic hydrolysate from brown alga Ecklonia cava. Food Chem Toxicol, 2006; 44: 1065-1074.
6. Li AH et al. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 2007; 102: 771-776.
7. Qi H et al. Antioxidant activity of different molecular weight sulfated polysaccharides from Ulvapertusa Kjellm (Chlorophyta). Appl Phycol, 2005; 17: 527-534.
8. Chkhikvishvili ID and Ramazanov ZM. Phenolic substances of brown algae and their antioxidant activity. Appl Bioche Microbiol, 2000; 36: 289–91.
9. Huang HL and Wang BG. Antioxidant capacity and lipophilic content of seaweeds collected from the Qingdao coastline. J Agric Food Chem, 2004; 52: 4993–97.
10. Lu F and Foo LY. Phenolic antioxidant components of evening primrose, In ASH, Ong, E Niki, L Packer (Eds.), Nutrition, Lipids, Health, and Disease Champaign. American Oil Chemists Society, 1995; 42: 86–95.
11. Duan XJ et al. Evaluation of Antioxidant property of extract and fractions obtained from a red alga *Polysiphoniaurceolata*. Food Chem, 2006; 95: 37-43.
12. Kuda T et al. Antioxidant properties of dried „kayamo-nori", a brown alga *Scytosiphonlomentaria* (*Scytosiphonales, Phaeophyceae*). Food Chemistry, 2005; 89: 617–22.
13. Miranda MS et al. Antioxidant activity of the microalga Spirulina maxima. Brazilian Journal of Medical and Biological Research, 1998; 31: 1075-9.
14. Murthy KNC et al. *In vivo* antioxidant activity of carotenoids from *Dunaliellasalina* – a green microalga. Life Sciences, 2005; 76: 1381–90.
15. Schwartz J and Shklar G. Regression of experimental hamster cancer by betacarotene and alga extract. Journal of Oral and Maxillofacial Surgery, 1987; 45: 510-5.
16. Fedkovic Y et al. Spirulina and cancer. Bulletin de l'Institut Oceano graphique, 1993; 12: 117-20.

17. Fernie AR et al. Leveraging metabolomics for functional investigations in sequenced marine diatoms. Trends Plant Science, 2012; 17: 395–403.
18. Vijayabaskar P and Shiyamala V. Antioxidant properties of seaweed polyphenol from Turinaria ornate (Turner). J, Agardh Asian Pacific Journal of Tropical Biomedicine, 2012; S90-S98.
19. Bhuvana P et al. In vitro antioxidant activity of methanolic extract of *Chlorella*. International Journal of Advanced Research, 2017; 5(11): 1465-1474.
20. Suganya V et al. In vitro antioxidant activity of microencapsulated and non-encapsulated astaxanthin. Asian Journal of Science and technology, 2017; 8(11): 6391-6404.
21. Taga MS et al. Chia seeds as a source of natural lipid antioxidants. Journal of American Oil Chemists' Society, 1984; 61: 928–931.
22. Chang CC et al. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 2002; 10: 178-182.
23. Prieto P et al. Anal Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Analytical Biochem, 1999; 269: 337-341.
24. Yen GH and Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural Food Chemistry, 1995; 43: 27–32.
25. Ruch RJ et al. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10: 1003.
26. Fadzai Boora et al. Evaluation of nitrite radical scavenging properties of selected zimbabwean plant extracts and their phytoconstituents. Journal of Food Processing, 2014: 1-7.
27. Oyaizu M. Studies on product of browning reaction prepared from glucoseamine. Japanese Journal of Nutrition, 1986; 44: 307-315.
28. Indu H and Seenivasan R. In Vitro Antioxidant Activity of Selected Seaweeds from Southeast Coast of India. International Journal of Pharmacy and Pharmaceutical Sciences, 1986; 5(2): 474-484.
29. Re R, Pellegrini N et al. Antioxidant activity applying an improved ABTS radical cation-decolorizing assay. Free Radical Biology and Medicine, 1999; 26: 1231-1237.
30. Winterbourn CC et al. The estimation of red cell superoxide dismutase activity, 1975; 85(2): 337-341.

31. Nishimiki M et al. The occurrence of super-oxide anion in the reaction of reduced phenazinemetho sulfate and molecular oxygen. Biochemistry and Biophysical Research and Communications, 1972; 46: 849-853.
32. Badmus JA et al. Evaluation of antioxidant, antimutagenic, and lipid peroxidation inhibitory activities of selected fractions of *Holarrhena floribunda* (G. Don) leaves. ACTA Biochimica Polonica, 2013; 60(3): 435-442.
33. Zargar M et al. Bioactive compounds and antioxidant activity of different extracts from *Vitexnegundo* leaf. Journal of Medical Plant Research, 2011; 5(12): 2525-2532.
34. Nakaya N et al. Cholesterol lowering effect of *Spiruline*. Nutrition Rep Int, 1998; 37: 1329–37.
35. Zhao M et al. Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chemistry, 2006; 98: 539–44.
36. Duan X et al. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules, 2007; 12: 759–71.
37. Li H et al. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem, 2009; 112: 454–60.
38. Rodriguez-Garcia I and Guil-Guerrero JL. Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. Food Chem, 2008; 108: 1023–1026.
39. Karthikeyan P et al. In vitro antioxidant acitivty of marine diatoms, IOSR Journal of Environmental Science. Toxicology and Food Technology, 2013; 5(2): 32-37.
40. Anantharaman P et al. Evaluation of antioxidant activities and total phenolic contents of different solvent extracts of selected marine diatoms. Indian Journal of Geo-Marine Sciences, 2015; 44(10): 1630-1636.
41. Anantharaman P et al. Antioxidant properties and total phenolic content of a marine diatom, *Navicula clavata* and green microalgae, *Chlorella marina* and *Dunaliella salina*. Advanced in Applied Science Research, 2013; l4(5): 151-157.
42. Uma R et al. Evaluation of in vitro antioxidant activities and anti proliferative activity of green microalgae, *Desmococcous olivaceous* and *Chlorococcum humicola*. Journal of Algae Biomass., 2011; 2: 82-93.
43. Manivannan K et al. Evaluation of antioxidant properties of marine microalgae *Chlorella marina*. Asian Pacific Journal of Tropical Biomedicine, 2012; 1-5.
44. Sivakumar K and Rajagopal SV. Radical scavenging activity of green algal species. Journal of Pharmaceutical Research 2011; 4: 723-725.

45. Lee SH et al. Antioxidant properties of tidal pool microalgae, *Halochlorococcum porphyrae* and *Oltamannsiellopsis unicellularis* from Jeju Island Korea. Algae., 2010; 25: 45-56.