ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 9, Issue, 03, pp.47846-47852, March, 2017*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**RESEARCH ARTICLE**

# ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ETHYL ACETATE EXTRACT FROM THE THALLUS OF *GRATELOUPIA DORYPHORA* COLLECTED FROM KOVALUM SEASHORE, TAMILNADU

**Kanimozhi, S. and *Sridhar, S.**

Department of Botany, Government Arts College, Thiruvannamalai, Tamil Nadu, India

**ARTICLE INFO**

***Article History:***
Received 17th December, 2016
Received in revised form
21st January, 2017
Accepted 17th February, 2017
Published online 31st March, 2017

***Key words:***

*Grateloupia doryphora,*
Ethyl acetate,
Antibacterial,
Antioxidant,
Flavonoids.

**ABSTRACT**

The objective of this study was to determine the phytochemical content, antibacterial activity and antioxidant activity of ethyl acetate extract of *Grateloupia doryphora*. The ethyl acetate extract of *G. doryphora* was evaluated fortotal phenolic, flavonoid contents, antibacterial (agar disc diffusion and broth dilution methods) and antioxidant (ABTS assay, lipid peroxidation, superoxide radical scavenging, nitric oxide radical scavenging and reducing power) activities. The results indicated that ethyl acetate extract of *G. doryphora* was effective in inhibiting the growth of Gram positive viz; *Staphylococcus aureus* and *Bacillus subtilis*, gram negative viz; *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* compared to antibiotic streptomycin. The ethyl acetate extract was found to be rich in total phenolic content, and high antioxidant activity as compared to Ascorbic acid. The presence of functional groups of active compounds was confirmed by Gas Chromatography mass spectroscopy (GSMS) analysis of ethyl acetate extract. It was concluded that all tested ethyl acetate extract of *G. doryphora* had antibacterial and antioxidant activities. These properties might be due to the presence of high total phenolic content or flavonoids. Hence, the ethyl acetate extract of thallus of *G. doryphora* represent a potential source of antibacterial and antioxidant compounds that may be used in food or pharmaceutical products.



**Citation: Kanimozhi, S. and Sridhar, S. 2017.** "Antioxidant and antibacterial activities of ethyl acetate extract from the thallus of Grateloupia doryphora collected from kovalum seashore, Tamilnadu", *International Journal of Current Research*, 9, (03), 47846-47852.

## INTRODUCTION

Marine algae are possible sources of bioactive secondary metabolites with possible for use in the development of new pharmaceutical drugs and many of these molecules have been confirmed to possess stimulating biological activities (Abdel-Raouf *et al.,* 2008). Marine algae were recorded to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances comprised alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols (Cabrita *et al.,* 2010) and marine macro-algae are considered as the definite producers of some bioactive molecule compounds with higher bio activity (Shimizu, 1996). Hence, they have strained great consideration newly (Cabrita *et al.,* 2010). Some of these bioactive compounds previously isolated from the families exhibited a wide spectrum of pharmacological activities such as enzyme inhibition, cytotoxic, antioxidant, feeding deterrent, antiflammatory and antimicrobial activities (Williamson and Carughi, 2010). Phenolic plant compounds have been found to exert diverse biological effects (Liu, 2004).

***Corresponding author: Sridhar, S.***
Department of Botany, Government Arts College, Thiruvannamalai, Tamil Nadu, India.

Nutraceutical properties of dietary plants are commonly considered in terms of their phenolic content. At the epidemiological level, plant polyphenols have been suggested to reduce the risk of cardiovascular diseases, neurodegenerative disorders and diabetes (Arts and Hollman, 2005). Their properties are related to the antibacterial and antioxidant. Actually, most of the antioxidative potential of plant foods, which could be beneficial to human health, is due to the properties of phenolic compounds. Reactive oxygen species (ROS) are produced in all aerobic cells as by-products of oxygen metabolism. When ROS generation overwhelms the cellular antioxidant capacity, oxidative stress ensues. Under these conditions, ROS can oxidize lipids, proteins and nucleic acids, ultimately leading to cell death or transformation. Phenolic compounds can act as reducing agents, free radical scavengers, hydrogen donators and inhibitors of pro-oxidative enzymes (Gawlik-Dziki *et al.,* 2012), thus participating in the prevention of DNA adduct formation and enhanced carcinogen elimination. However, they can also exert chemopreventive effects through interference with ROS, which act as secondary messengers in signaling pathways crucial for cancer cell proliferation and invasion (Dai and Mumper, 2010). The blade of *Grateloupia doryphora* is purplish-red, with a characteristic emerald-green coloration at the base and usually scattered pale areas on the fronds of mature thalli. The blade, 200–450 µm thick, has a short stipe and smooth margins. Marginal

proliferations usually develop only in eroded areas or as a result of damage or grazing. Generic delimitation within the red algal family Halymeniaceae is in a state of flux. Approximately 21 genera have traditionally been characterized on the basis of various gross morphological characters relating to thallus size, form, texture, and degree of branching. The aim of this study was to assess the nutraceutical potential of *G.doryphora* in the context of the bio availability of their phenolic compounds. For this purpose, present study analyzed the phenolic content of *G. doryphora* and estimated its combined bioactivity, using an experimental model based on in vitro study of antioxidant and antibacterial activity.

## MATERIALS AND METHODS

### Collection of Algae

Algal materials were collected from the littoral zone of the Kovalum region between (0.2–2.5 m depths) along the East coast, Near Chennai, Tamilnadu. The collected algal specimen were stored in plastic bags and transported to the laboratory under iced conditions. The samples were primarily washed thoroughly with sea water to remove sand and any adhering substance and then washed thoroughly with fresh water to remove salts, and stored at 20 °C until compound extraction. The algal species were identified based on the schemes reported in the literature (Smith, 1944; Bold, 1978; Aleem, 1993).

### Extraction of selected algal species

The collected fresh algae subsequently washing with distilled water for several times, the algal specimen were again washed with 5% ethanol to remove any epiphytes or salts. The samples were subjected to air drying under the shade. After drying algal specimens were ground by an electrical mixer until they became a powder. Then the powdered algal specimen were stored in a dark place, and subjected to extraction methods. Extraction of powdered algal specimens was done using ethyl acetate. Aliquots of 25 g of the powdered algal samples were soaked in 250 ml of the solvents for 24 h. Later the soaked samples were homogenized in an electric blender along with the solvents at room temperature, filtered, and concentrated under reduced pressure using a rotary evaporator.

### Reducing power determination

Fe (III) reduction is often used as an indicator of electron-donating activity, an important mechanism for antioxidant action (Nabavi *et al.,* 2008).The reducing power of the ethyl acetate extract from *G. doryphora* was determined by spectrophotometric method of Yen and Chen (1995). The acetate extract (5-20µl) was mixed with 2.5 ml of 0.2 M Potassium phosphate buffer (pH-6.6) and 2.5 ml of 1% Potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 minutes, then rapidly cooled, mixed with 2.5 ml of trichloroacetic acid and centrifuged at 5000 rpm for 3 minutes. An aliquot (2.5ml) of supernatant was diluted with distilled water (2.5ml) and 0.5 ml of 0.1% Ferric chloride was added and allowed to stand for 10 minutes. The absorbance was read spectrophotometrically at 700 nm. Increased absorbance indicates increased reducing power. Vitamin C was used as positive control.

### ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

ABTS radical scavenging activity of ethyl acetate extract from *G. doryphora* was followed by Re *et al.* (1999). ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium per sulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of flavonoid rich fraction. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

ABTS Scavenging Effect (%) = $[(A_{0-}A_1/A_0) \times 100]$

Where $A_0$ is the absorbance of the control reaction and $A_1$ is the absorbance of flavonoid rich fraction.

### Inhibition of lipid peroxidation activity

Lipid peroxidation induced by $Fe^{2+}$ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.* 1979. The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of ethyl acetate extract from *G. doryphora* in a final volume of 0.5 ml. The experimental mixture was incubated at 37$^o$C for 1 hour. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard. Inhibition of lipid peroxidation (%) by the flavonoid rich fraction was calculated according to 1-(E/C) X 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

### Superoxide radical scavenging assay

This assay was based on the capacity of the flavonoid rich fraction to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971) in the presence of the riboflavin-light-NBT system, as described earlier (Tripathi and Pandey, 1999; Tripathi and Sharma, 1999). Each 3 ml reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of ethyl acetate extract from *G. doryphora*. It was kept visible in fluorescent

light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark served as blanks. The percentage inhibition of superoxide radical activity was measured by comparing the absorbance of the control with test sample solution.

% Super oxide radical scavenging capacity= $[(A_0-A_1)/A_0] \times 100$

Where $A_0$ was the absorbance of control and $A_1$ was the absorbance of flavonoid rich fraction.

**Nitric oxide radical scavenging activity**

Nitric oxide scavenging ability of acetate extract were measured according to the method described by Olabinri *et al*., 2010. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of ethyl acetate extract from *G. doryphora* and incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the experimental sample was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The percentage of inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity= $[(A_0-A_1)/A_0] \times 100$

Where $A_0$ was the absorbance of control and $A_1$ was the absorbance of flavonoid rich fraction.

**Antibacterial Properties**

**Bacterial strains**

Bacteria used for the assessing antibacterial activities were Gram positive viz; *Staphylococcus aureus* MTCC 29213, *Bacillus subtilis* MTCC 1771, gram negative viz; *Pseudomonas aeruginosa* MTCC 2488, and *Klebsiella pneumoniae* MTCC 25922. The bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh-160036, India. All bacterial strains were sub cultured on nutrient agar medium, incubated at 37 °C for 24 h and stored at 4 °C in refrigerator to maintain stock culture.

**Antibacterial assay**

Antibacterial activity was assessed using disc diffusion method followed by Sathyabama *et al*., 2011. The petriplates were prepared with 20 ml of sterile nutrient agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 min. The test was conducted in four different concentrations of the ethyl acetate extract from *G. doryphora* (5, 10, 15 & 20 µl/ml) and treated discs (Whatman No.1 filter paper was used to prepare discs) were air dried. The treated discs were placed on the surface of the medium and incubated at room temperature for 24 h. The relative inhibition of organisms to the flavonoid rich fraction was indicated by a clear zone of inhibition around the discs. It was then detected, measured and documented in millimeters with three replicates.

**GC–MS analysis**

The flavonoid fraction was analyzed by GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan) equipped with 30- mX 0.25 mm DB-5MS column (Agilent Technologies, J& W Scientific Products, Folsom, CA). The carrier gas was helium. The temperature program was set as follows: 100 °C hold for 5 min, raised at 4 °C/min to 280 °C, and hold for 5 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The ion source and interface temperatures were set at 200 and 250 °C, correspondingly. The mass range was scanned from 50 to 900 amu. The control of the GC–MS system and the data peak processing were controlled by Shimadzu's GC–MS solution software, version 2.4. Compound identification was confirmed based on the relative retention time and mass fragmentation pattern spectra with those of standards and the NIST147. LIB. Database of the GC–MS system (Shimadzu).

**Statistical analysis**

The impact of the ethyl acetate extract of *G. doryphora* on its antioxidant activity was measured by the ABTS assay, lipid peroxidation, superoxide scavenging, metal chelating and nitric oxide radical were determined using one-way analysis of variance (ANOVA). Likewise, Duncan's post hoc test was applied, so as to determine the statistically significant different values. All statistical handling was performed using SPSS software, v. 14.0 (SPSS, Chicago, Ill., U. S. A.).

## RESULTS AND DISCUSION

**Phytochemical screening of *Grateloupia doryphora***

The phytochemical screening revealed the presence of Flavonoids (catechins and flavones), terpenoid, polyphenol, tannin and absence of alkaloids, glycoside and saponin (Table-1). Similarly, seaweeds contain glycosides, carbohydrates, tannins, steroids, terpenoids, phytosterols (Manchu *et al*., 2014). Phenolic compounds are commonly found in plants, including and have been reported to have a wide range of biological activities including antioxidant properties. These secondary metabolites possess wide variety of antioxidant, antibacterial, anticancer activities and hence could be effective potential antioxidant agents due to selective anticancer effect and low toxicity in vivo (Kuda and Ikemori, 2009).

**Table 1. Phytochemical screening of aqueous extracts of *Grateloupia doryphora***

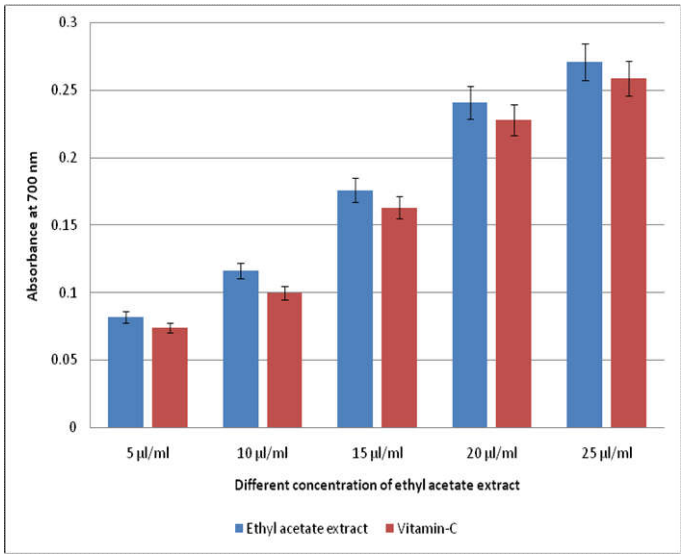
| S.No. | Constituents | Aqueous extract of *Grateloupia doryphora* |
|---|---|---|
| 1. | Alkaloids<br>-Dragendroffs reagent<br>- Mayer's test | <br>-<br>- |
| 2. | Flavonoids<br>- Alkali test<br>- Lead acetate test | <br>+<br>+ |
| 3. | Polyphenols<br>-Ferrozine test | <br>+ |
| 4. | Terpenoids<br>-Salkowski test | <br>+ |
| 5. | Tannins<br>-$Fecl_3$ Test | <br>+ |
| 6. | Glycosides<br>-Keller-Killani test | <br>- |
| 7. | Saponins<br>-Froth test | <br>- |

-- = Negative (absent); + = Positive (present)

### Effect of ethyl acetate extract from *Grateloupia doryphora* on reducing power determination

Intimate the reducing power assay, the presence of reductants as antioxidants in tested samples would result in reducing $Fe^{3+}$ferricyanide complex to the ferrous form. The $Fe^{2+}$ can therefore be monitored by measuring the formation of Perl's prussian blue at 700 nm. Fig-1 shows the reducing powers of *G. doryphora* ethyl acetate extract, and standard as Vitamin C.

The reducing power increased with the concentration of the *G. doryphora* extract to a certain extent, then leveled off with further increase in the concentration than Vitamin -C. This statement accordance with previous studies, in which *Laurencia* spp., although a good source of biologically active secondary metabolites (Zubia *et al*., 2007). Considering the extensive range of bioactivity of *Polysiphonia* extracts (Abbott, 1999), it should be considered a hopeful source of not only natural antioxidants, but also a wide range of bioactive compounds.

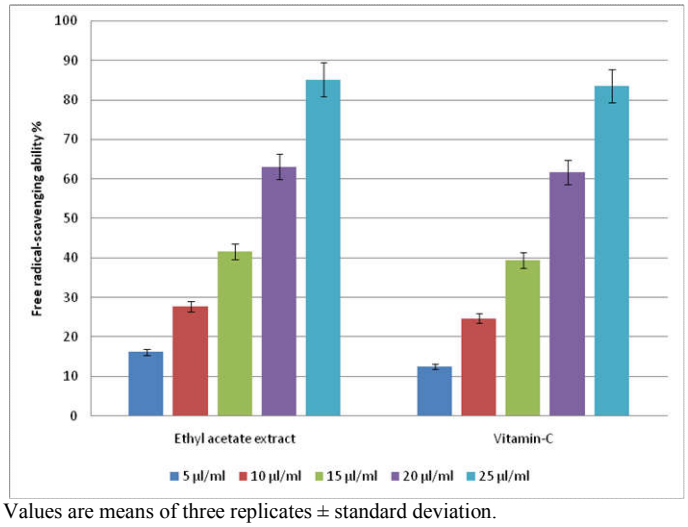



Values are presented as mean ± standard deviation of three individual determinations.

**Fig 1. Effect of ethyl acetate extract from *Grateloupia doryphora* on reducing power determination**

### Effect of ethyl acetate extract from *Grateloupia doryphora* on ABTS radical activity

Ethyl acetate extract of *G. doryphora* exhibited a powerful scavenging activity for ABTS radical cations in a concentration dependent manner. Fig-2 showing a direct role in catching free radicals. Maximum inhibition was observed with the *G. doryphora* extract ranges from 16.25 to 85.28% at 5-25 µl/ml than positive control vitamin-C. The ABTS radical cation decolorization assay can measure the relative antioxidant ability to scavenge the radical ABTS as compared with Vitamin-C, and is an excellent tool for determining the antioxidant capacity of hydrogen-donating antioxidants. The polyphenol antioxidant activity is due to the chemical structure and ability to donate/accept electrons, thereby delocalizing the unpaired electron within the aromatic structure (Ross and Kasum, 2002). According to Matanjun *et al.* (2008), radical scavenging capacity of red seaweed extracts may be mostly related to their phenolic hydroxyl groups. Seaweed contains a novel antioxidant compound that controls free radical formation from metabolic reactions.



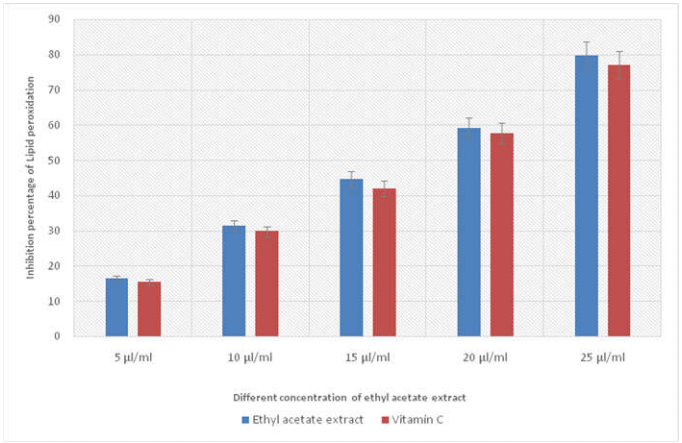

Values are means of three replicates ± standard deviation.

**Fig 2. *Grateloupia doryphora* ethyl acetate extract expressed as capacity to bleach the stable ABTS radical**

### Inhibition of lipid peroxidation by ethyl acetate extract of *Grateloupia doryphora*

Ethyl acetate extract of *G. doryphora* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed with total *G. doryphora* extract with inhibition percentage 16.55 to 79.79 at 5 to 25µg/ml then Vitamin-C (Fig-3). This inhibition of lipid peroxidation possibly either due to chelation of Fe or by corner of the free radicals. Iron also is playing a major role for the formation of lipid peroxidation in the body. Similarly, Jun Mori *et al*., 2003 reported that *in vitro* study of methanol, chloroform/ methanol (3:1) extract and ethyl acetate fraction of *Sargassum micracanthum* inhibited lipid peroxidation in rat liver homogenates. These inhibitions were stronger than vitamin C and E.

The present study associates fit with earlier studies. Zubia *et al*. (2007) reported that ethyl acetate fractions are the major seaweed fractions protecting the principle antioxidative mechanisms that inhibits lipid peroxidation. These finding inhibition of lipid peroxidation may be due to the presence of polyphenolic antioxidants to interrupt free-radical chain reaction by providing a proton to fatty acid radicals to terminate chain reactions.

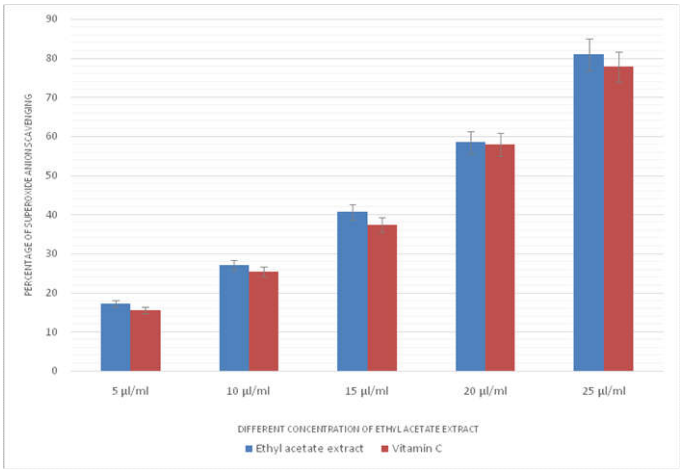



Data are the mean values of triplicate and expressed as mean ± standard deviation.

**Fig 3. Inhibition of lipid peroxidation by ethyl acetate extract of *Grateloupia doryphora***

### Superoxide anion scavenging activity of ethyl acetate extract of *Grateloupia doryphora*

Superoxide radicals by photochemical decrease of nitro blue tetrazolium (NBT) in the occurrence of a riboflavin-light-NBT system, which is one of the standard methods. The total fractions ethyl acetate extract of *G. doryphora* exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner (Fig-4). The *G. doryphora* extract had highest Superoxide radicals scavenging percentage 80.99 at 25µg/ml and the Vitamin-C was lesser potent with 77.85 value at 25µg/ml. Removal of superoxide in a concentration dependent manner by any solvent fractions may be attributed to the direct reaction of its phytomolecules with inhibition of the enzymes. These results are in agreement with data obtained previously by the EtOAc fraction of *Eisenia bicyclis* showed the highest level of activity; the $H_2O$ fraction showed the lowest. The results clearly showed that ethyl acetate extract from *G. doryphora* scavenging activity of superoxide anion may be potential antioxidant agents.




Data are the mean values of triplicate and expressed as mean ± standard deviation.

**Fig 4. Superoxide anion scavenging activity of ethyl acetate extract of *Grateloupia doryphora***

### Nitric oxide scavenging assay of ethyl acetate extract of *Grateloupia doryphora*

Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane.

In present study ethyl acetate extract of *G. doryphora* showed Nitric oxide scavenging activity. Inhibition increased with increasing concentration of the ethyl acetate extract of *G. doryphora*. Virtuous result was observed in *G. doryphora* ethyl acetate extract with scavenging ranges 72.85±0.91at 25µg/ml than compared with at 25µg/ml for vitamin- C which served as positive control (Fig-5). Nitric oxide scavenging due to extracts contain poly phenol compounds; free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage. The results of the present study are contradictory to Monsuang *et al.,* (2009) who reported that green seaweeds showed significantly higher phenolic content and antioxidant activities than red seaweeds.

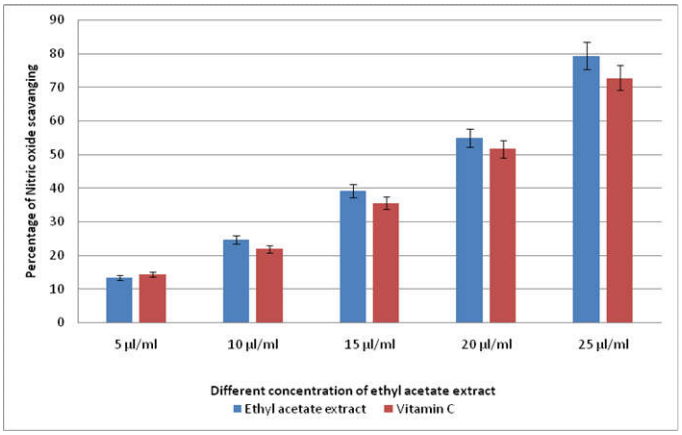



**Fig 5. Nitric oxide scavenging assay of ethyl acetate extract of Grateloupia doryphora**

### Anti-bacterial activity of ethyl acetate extract of *Grateloupia doryphora* tested against pathogenic bacteria

Antibacterial activities of the ethyl acetate extract of *G. doryphora* against the tested organisms are shown in Table-2. The algae differ in their activities against the micro-organisms tested. Ethyl acetate extract of *G. doryphora* showed maximum antibacterial activity against *Pseudomonas aeruginosa, B.subtilis, and Staphylococcus aureus* than *K. pneumoniae.* Highest antibacterial activity was observed with ethyl acetate extract of *G. doryphora* against *P. aeruginosa,* (19.3 mm) where in the case of lowest activity was observed against *Bacillus subtilis* with the inhibition zone of 16.3 mm. Results obtained in the current investigation revealed that the algae extract possesses potential antibacterial activity against entire tested organisms.

**Table 2. Antibacterial activity of the *G. doryphora* ethyl acetate extract by disc diffusion method**

| Pathogenic organism | Different concentration of ethyl acetate extract of *G. doryphora* | | | |
|---|---|---|---|---|
| | 5µl/ml | 10µl/ml | 15µl/ml | 20µl/ml |
| *Staphylococcus aureus* | 8.8±0.49 | 12.7±0.56 | 15.2±0.56 | 17.4±0.41 |
| *Bacillus subtilis* | 8.1±0.20 | 11.00±0.80 | 13.4±0.45 | 16.3±0.41 |
| *Klebsiella pneumoniae* | 10.6±0.51 | 13.5±0.50 | 15.7±0.10 | 18.4±0.030 |
| *Pseudomonas aeruginosa* | 8.7±0.32 | 12.7±0.52 | 15.6±0.37 | 19.3±0.39 |

**Table-3. Phyco-constituent of *Grateloupia doryphora***

| S.No. | Compound | Retention Time(min) | Molecular weight |
|---|---|---|---|
| 1 | Pentanedione (2,4-di-t butylphenyl) mono ester | 12.52 | 646.92 |
| 2 | Hexadecane | 13.43 | 226.44 |
| 3 | Octadecanoic acid methyl ester | 19.1 | 294.47 |
| 4 | octadecane, 1-[2-hexadecyloxy ethoxy | 20.48 | 532.97 |
| 5 | octadecane | 25.48 | 254.49 |

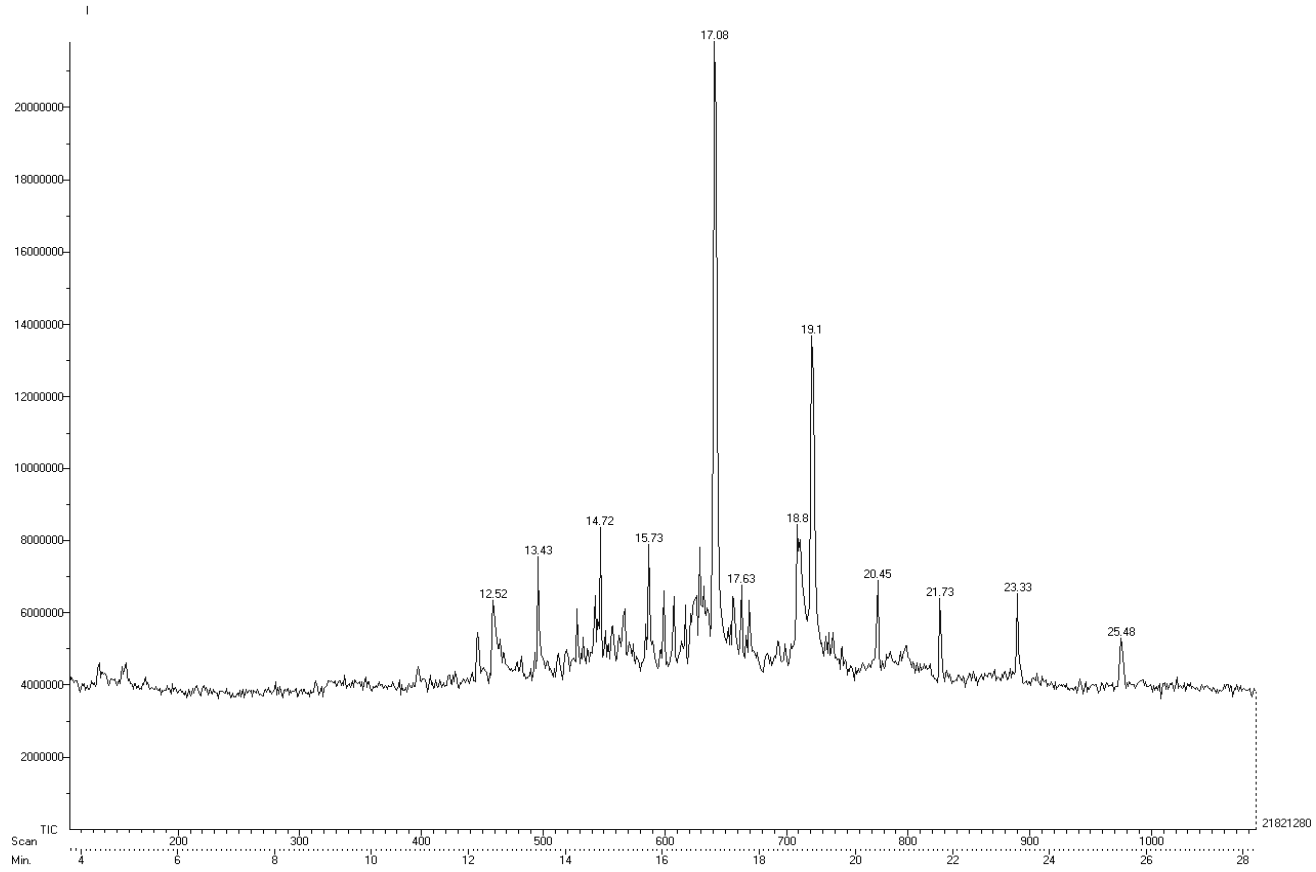



**Fig.6. Phyco-constituent analysis by GC–MS from ethyl acetate extract of *Grateloupia doryphora* (Consolidated retention Time peaks)**

Generally algae extracts are identified polyphenols, notably gentisic acid, (+) catechin and (−) epicatechin (Lopez *et al*., 2015). Since, that the antimicrobial activity of polyphenols is already recognized, the activity against bacteria of the extract can be supposed to be due to these compounds. The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

**Analysis of phyco-constituent of *Grateloupia doryphora* by GC–MS**

The chemical constituents of the *G. doryphora* analyzed by GC–MS and confirmed highest polyphenol compounds and exhibited the strongest antioxidant activity (Table-3). It was to determine its chemical composition that may contribute to these activity. The GC–MS analysis showed a variety of phyto-constituent (Fig-5).

**Conclusion**

In conclusion the results of the present investigation on selected species of marine algae *Grateloupia doryphora* indicated scope for originating biologically active compounds which are effective antioxidant and inhibiting the growth of the pathogenic bacteria both Gram-positive and Gram-negative. Further, the *G. doryphora* has potential to return pharmaceutically useful seaweeds which can be harnessed for the development of drugs for use in management of human pathogens and many ageing related human degenerative diseases. There is great scope for further investigations toward drug development.

## REFERENCES

Abbott I.A. 1999. Marine Red Algae of the Hawaiian Islands. Bishop Museum Press; Honolulu, HI, USA.

Abdel-Raouf, N., Ibraheem, I.B.M., Abdel-Tawab, S. and Naser, Y.A.G. 2011. Antimicrobial and anti-hyperlipidemic activities of isolated quercetin from *Anabaena aequalis*. *J. Phycol*., 47: 955–962.

Aleem, A.A. 1993. Egyptian Books House; Egypt. The Marine Algae of Alexandria.

Arts, I.C. and Hollman, P.C. 2005. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr*., 81 (1): 317–325.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. *Anal Biochem*., 44:276-87.

Bold, H.C. 1978. Prentice Hall, Inc.; New-Jersey 07632. "Introduction to the Algae" Structure and Reproduction.

Cabrita, M., Vale, C. and Rauter, A. 2010. Halogenated compounds from marine algae. *Mar. Drugs*, 8: 2301–2317.

Dai, J. and Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15 (10): 7313–7352.

Gawlik-Dziki, U., Swieca, M. and Sugier, D. 2012. Enhancement of antioxidant abilities and the lipoxygenase and xanthine oxidase inhibitory activity of broccoli sprouts by biotic elicitors. *Acta Sci. Pol. – Hortoru*. 11 (1): 13–25.

Jun, M., Takayuki, M., Satoshi, T., Chika, H. and Haruo, S. 2003. Inhibitory activity on lipid peroxidation of extracts from marine brown alga. 17 (5): 549–551.

Kuda, T. and Ikemori, T. 2009. Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from

macro algal beach casts in the Noto Peninsula, Ishikawa, Japan. *Food. Chem.,* (112): 575-581.

Liu, R.H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutr*., 134: 3479–3485.

Lopez, A., Rico, M. and Santana-Casiano J.M. 2015. Gonzalez-Davila M. Phenolic profile of *Dunaliella tertiolecta* growing under high levels of copper and iron. *Environ. Sci. Pullut. Res. Int*., 22:14820–14828.

Manchu, N., Melpha, Y. and Edwin James, J. 2014. Phytochemical investigation of three species of *Ulva* from Rasthacaud Coast, Tamil Nadu, India. *J. Chem. Pharmaceut. Res.,* 6(8):570-574.

Matanjun, P., Suhaila, M., Noordin, M. M., Kharidah, M. and Ming, C.H. 2008. Antioxidant activities and phenolic content of eight species of seaweeds from north Borneo. *J. Appl. Phycol*., 20: 367- 373.

Monsuang, Y., Nongporn, H. T. and Wutiporn, P. 2009. Antioxidant Activities of Four Edible Seaweeds from the Southern Coast of Thailand. *Plant Foods for Human Nutri.*, 64(3): 218–223.

Nabavi, S. M., Ebrahimzadeh, M. A., Nabavi, S. F., Hamidinia, A. and Bekhradnia, A. R.2008. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline,*2: 560-567.

Ohkawa, H., Ohisi, N. and Yagi, K. 1979. Assay for lipid peroxides in animals tissue by thiobarbituric acid reaction. *Anal. Biochem*., 95:351-358.

Olabinri, B. M., Odedire, O. O., Olaleye, M. T., Adekunle, A. S., Ehigie, L. O. and Olabinri, P. F. 2010. In vitro evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. *Res. J. Biol. Sci*., 5: 102-105.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. *Free Rad. Biol. Med*., 26:1231-1237.

Ross, J.A. and Kasum, C.M. 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Ann. Rev. Nutr*., 22: 19–34.

Sathyabama, S., Jayasurya Kingsley, S., Sankaranarayanan, S. and Bama, P. 2011. Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens*: identification and mode of action of terpenoid compound as antibacterial. *Inter. J. Phar. Pharmce. Sci.,* 4: 557-564.

Shimizu, Y. 1996. Microalgal metabolites: a new perspective. *Annu. Rev. Microbiol*., 50: 431–465.

Smith, G. M. 1944.Marine algae of the Monterey Peninsula. Reprinted from Madrono VII (7), pp. 226–231.

Tripathi, Y. B. and Pandey, E. 1999. Role of alcoholic extract of shoot of *H. perforatum* (Lim) on LPO and various species of free radicals in Rats. *Ind. J. Exp. Biol*., 37:567-571.

Tripathi, Y. B. and Sharma, M. 1999. The Interaction of *R. cordifolia* with iron redox status: mechanistic aspects in FR reactions. *Phytomedicine,* 6: 51-57.

Williamson, G. and Carughi, A. 2010. Polyphenol content and health benefits of raisins. *Nutr. Res*., 30 (8): 511–519.

Yen, G.C. and Chen, H.Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem*. 46:849-854.

Zubia, M., Robledo, D. and Freile-Pelegrin, Y. 2007. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *J. Appl. Phycol*., 19:449–458.

*******