

## Original Research Article

### Phytochemical Screening by GCMS and *Invitro* Antioxidant Activity of *Griffithsia pacifica Kylin*

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## A B S T R A C T

### Keywords

Red algae,  
*Griffithsia*  
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Antioxidant,  
DPPH, Ascorbic  
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Nitric oxide  
radical  
inhibition assay

Under biotic/abiotic stresses, the red alga reportedly releases massive amounts of H<sub>2</sub>O<sub>2</sub> into the surrounding seawater. *Griffithsia Pacifica Kylin*(GPK)a marine red algae have high content of steroid, phenol, alkane, bromine and iodine. This study was designed to examine the GC-MS and *invitro* antioxidant activities of ethanol extract of GPK. Considerable differences were observed both in total phenolic content (TPC) and antioxidant activities of the hydroxylases evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Free radical scavenging assay, Nitric oxide radical inhibition assay, Ascorbic acid Assay, Superoxide anion scavenging activity, Lipid peroxidation assay, Reducing Power, Hydroxyl Radical Scavenging Activity. The findings of the present study suggested that GPK could be potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

## Introduction

Marine macro algae have a long history of use as food and folk medicine in Asia, but are much less common in Europe and North America. In recent years, there is increasing interest in the search for naturally occurring compounds with antioxidant activity as alternatives to synthetic products. Aquatic algae are also a rich source of natural antioxidants (Duan XJ, Li XM, Wang et al 2007). They are excellent source of biologically active phytochemicals, which include carotenoids, phycobilins, fatty

acids, polysaccharides, vitamins, sterols, tocopherol, and phycocyanins among others. Many of these compounds have been recognized to possess biological activity and hence beneficial for use in human and animal healthcare (Gamal, guven et al 2010)

Some of the potential benefits include control of hyperlipidaemia, tumour, and obesity (Vishwamodia, Rakesh Somani et al 2009). Oxidative stress is an important factor in the genesis of much pathology,

from cancer to cardiovascular and degenerative diseases. (Biswas K, Chatopadhyay I, Banerjee et al 2002). In order to protect the body against the consequences of oxidative stress, an efficacious approach consists in improving the antioxidant nutrition. In this regard, scientific studies have shown that the synergistic action of a wide spectrum of antioxidants is better than the activity of a single antioxidant, and that antioxidants from natural sources (primarily foods) have a higher bioavailability and therefore higher protective efficacy than synthetic antioxidants(Gey 1998. Oyaizu,1986)

Focusing our attention on natural and bio-available sources of antioxidants, we undertook to investigate the antioxidant properties of the *Griffithsia Pacifica Kylin* (*GPK*) a fresh water unicellular red alga that spontaneously grows in water sources and that is consumed as a nutrient-dense food source and for its health-enhancing properties(Jensen et al., 2000,2001; Pugh and Pasco, 2001; Pugh et al., 2001).

*Griffithsia Pacifica* has the following physical characteristics: an agarophyte, produces hydrocolloid agar in its cell walls, is a multicellular thallus with apical growth, has tetra sporangia, has a filamentous gonimoblast, has pit connections, cells have multiple nuclei and plastids, triphasic alternation of generations life history, a red algae, eukaryotic cells without flagella and centrioles, using floridean polysaccharides as food reserves, phycobiliproteins as accessory red pigments, chloroplasts lacking external endoplasmic reticulum and containing un stacked thylakoids, multicellular organisms, have a cell wall made of cellulose, obtain energy from sun typically with chlorophyll, sexual or asexual reproduction, modular and indeterminate growth, alternation of generations.

## Materials and Methods

### Collection of sample

The algae sample (*Griffithsia Pacifica Kylin* Photographs 1) was collected as entangled specimens from a bottom trawl fish net operated off Manoli and Hare Islands of Mandapam group of Islands, Gulf of Mannar at Rameswaram. It was collected by bicatching method. The samples were placed inside sterile ethyl polythene bags under water and transferred to the lab aseptically in ice boxes.

### Extraction of marine red algae

The whole red algae was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol in sox let's apparatus to 60°c. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators.

### GC-MS analysis of ethanol extract of *Griffithsia pacifica kylin* for identification of chemical compounds

The identification of chemical composition of ethanol extract of marine red algae was performed using a GC-MS spectrograph fitted with electron impact mode. The ethanol extract (2 $\mu$ l) of Red algae was injected with a Hamilton syringe to the GC-MS manually for column – Elite 5MS (5% Diphenyl/95% dimethyl polysiloxane). 30x0.25mmx0.25 $\mu$ mdf.

Equipment used GC Claus, 500 Perkin Elmer, Carrier gas was 1ml/min, split 10:1, Detector used for GC-MS was Mass detector, Turbo mass gold Perkin Elmer. Software was Turbo mass 5.2.

## Identification of phytocompounds

Interpretation on mass-spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

The studies on the active principles in the *Griffithsia Pacifica Kylin* whole red algae ethanol extract by GC-MS analysis clearly showed the presence of fourteen compounds (Tab-1).The active principles with their retention time (RT) molecular formula, molecular weight (MW), and concentration (peak area%)are presented in Table-1.The GC-MS chromatogram of peak of the compounds detected was shown in Figure-1.Chromatogram GC-MS analysis of the ethanol extract of *GPK* Showed the presence of major peaks and the components corresponding to the peaks were determined as follows. The First set up peaks was determined to be 1- Iodo-2-methylnonane with 7.41% of spectral area. The second peaks indicated to be pentanal, 2, 4-dimethyl- (RT=11.24min) with 3.10% of peak area. Following this an area up 0.96% cowered in the mass spectrum by n-Decanoic acid, 2-methylat (RT-12.15 min). The next peaks considered to be 5.49% of Nonanoic acid (RT=12.92min). The fifth peak indicated to be 4, 8 Dioxaspiro (2, 5) oct-1-ene, 6, 6-dimethyl- (RT=14.70 min) with 2.74% of peak area. The sixth peak (RT=16.25) were determined to be Hexadecanoic acid, 2-oxo-methyl ester with 1.78 peak area. while the seventh peak (RT=18.00 min) were attributed as 2-Bromononane with peak area 1.83, the eighth peak (RT=19.38) Tridecane, 6-

methyl-, the next peak (RT=19.98) Didodecyl phthalate with spectral peak 4.01, the tenth one peak (RT=22.14) Heptadecane,9-hexyl- with peak area1.02, while the next comprises (RT=23.67) Trifluoroacetyl-lavandulol with peak area 2.74, the twelfth one Cholesta 4,6-dien-3-ol,(3a) with (RT=24.43) with peak area of 11.58, the next (RT=24.69) of 5a-Androstan-16-one,cyclic ethylene mercaptole with peak area 2.64, and the final one (RT=27.65) Androstan-17-one, 3-ethyl-3-hydroxy-(5-Alpha) with peak area of 52.31.

Among the identified phyto compounds Hexadecanoic acid 2-oxo-methyl ester, Cholesta-4, 6-dien-3-ol, 5a Androstan-16-one, cyclec ethylene mercaptole have the property of anti-oxidant and antimicrobial activities. , Cholesta-4, 6-dien-3-ol, 5a-Androstan-16-one, cyclec ethylene mercaptole, Androstan-17-one, 3 ethyl-3-hydroxy-(5-Alpha) have Diuretic activity. Hexadecanoic acid has hypocholesterolic activity. (Dr.Duke's phytochemical and Ethano botanical database).

## Antioxidant-non enzymatic assay

### Freeradicalscavenging assay(DPPH):

The scavenging activity of DPPH free radicals by different algae extracts was determined according to the method reported by Gyamfi.(Gyamfi et al., 1999) Fifty micro liters of the algae extract in ethanol, yielding 100 µg/ml in each reaction, was mixed with 1 ml of 0.1 mM DPPH in ethanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Ethanol (50 µl) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, reading the absorbance at 517nm. Butylated Hydrody

Toluene(BHT) and a-toco pherol were used as controls. The percent inhibition was calculated from the following equation: The hydro alcoholic extract of *GPK* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC<sub>50</sub>) at a concentration of 1.5 $\mu$ g/ml. The result was mentioned in figure 2. The IC<sub>50</sub> value of the extract was found to be lesser than the standard, vitamin C (IC<sub>50</sub> 3.0  $\mu$ g/ml).

$$\text{\% Inhibition} = (\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance of control} \times 100$$

**Estimation of ascorbic acid:** Ascorbic acid was estimated by the method of Roe and Kuether (Roe and Kuether (1946). Ascorbic acid was converted to dehydro ascorbic acid by mixing with acid washed norit and then coupled with 2,4-dinitrophenyl hydrazine (DNPH) in the presence of thio urea, a mild reducing agent. The coupled di-nitro-phenyl hydrazine was converted in to an orange-red colored compound which when treated with sulphuric acid was read at 520nm. A set of standards containing 20-100 $\mu$ g of ascorbic acid were taken and processed similarly along with a blank containing 2.0 ml of 4% tri-chloro-acetic acid. The color developed was read at 540 nm. The values of ascorbic acid were expressed as mg/dl. Figure.2.

**Nitric oxide radical inhibition assay:** Nitric oxide radical inhibition can be estimated by the use of GriessIllosvoy reaction (Garrat, 1964). In this investigation GriessIllosvoy reagent was modified by using naphthyl ethylene diamine-dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin used as a standard. The scavenging of nitric oxide by

algae extract was increased in a dose-dependent manner as illustrated in figure 3. At concentration of 116.0  $\mu$ g/ml of extract 50% of nitric oxide generated by incubation was scavenged. This IC<sub>50</sub> value of extract found to be lesser than the standard rutin.

**Estimation of lipid hydroperoxide:** Tissue lipid hydro peroxide was estimated by the method of Jiang *et al.* (Jiang Cornish M.L., Garbary D.J *et al*, 1992.) In this method, oxidation of ferrous ions ( $Fe^{2+}$ ) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560nm. Lipid hydro peroxide were expressed as mmoles/100 mg extract. Activity of *GPK* extract against non-enzymatic lipid peroxidation in rat liver microsomes has been shown in figure 5. The extract showed inhibition of peroxidation effect in all concentrations which showed 50% inhibition effect at 104.0  $\mu$ g/ml. The extract inhibition value was found to be lesser than the standard, vitamin E (IC<sub>50</sub> 120.5  $\mu$ g/ml).

**Superoxide anion scavenging activity:** Measurement of superoxide anion scavenging activity of *GPK* was done based on the Nishimiki method (Nishimiki *et al.*, 1972). The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The superoxide anion derived from dissolved oxygen by Phenazinemethosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease the absorbance at 560 nm with the *GPK* extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in figure 4, the *GPK* extract as well as curcumin showed the scavenging activity; IC<sub>50</sub> values, 4.7  $\mu$ g/ ml and 5.84 $\mu$ g/ml, respectively.

**Reducing power:** The reducing power of *GPK* was determined according to the Oyaizu method (Oyaizu, 1986). Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxy toluene used as a standard. Figure 7 shows the reductive capabilities of the *GPK* extract compared to butylated hydroxy toluene. The reducing power of extract of *Griffithsia Pacifica Kylin* was very potent and the power of the extract was increased with quantity of sample. The *GPK* extract could reduce the most Fe<sup>3+</sup> ions, which had a lesser reductive activity than the standard of butylated hydroxy toluene.

**Determination of total phenolic compounds:** Total soluble phenolic in the aqueous extract of *GPK* were determined with Folin-Ciocalteu reagent according to the standard method using pyro catechol as a standard (Slinkard et al., 1977 Slinkard K, Singleton VL). The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the *GPK* determined as microgram of pyro catechol by using an equation that was obtained from Gulcin (Gulcin Peirce A. Duh et al Duh PD, et al., 2002) The equation is, Absorbance = 0.001 x Pyro catechol ( $\mu$ g) + 0.0033. The total phenolic contents of hydro alcoholic extract of *Griffithsia Pacifica Kylin* were 0.0589  $\mu$ g pyro catechol equivalent / mg.

**Hydroxyl radical scavenging assay:** The assay was performed as described by Halliwell method (Halliwell Mukherjee PK et al., 1987) with minor changes. All solutions were prepared freshly. After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation was measured by the TBA. Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control (IC<sub>50</sub> 160.0  $\mu$ g/ml). To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of

Ferric-EDTA together with H<sub>2</sub>O<sub>2</sub> and ascorbic acid. When the *GPK* extract were incubated with the reaction mixture, it could prevent the damage against sugar. The results are shown in figure 6, the concentrations of 50% inhibition were found to be 27.0  $\mu$ g/ml and 32.5  $\mu$ g/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

## Results and Discussion

There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (Rose WM, Creighton MO, Stewart DHPJ, Sanwal M, et al 1982). Several concentrations ranging from 0.5  $\mu$ g/ml - 150  $\mu$ g/ml of the alcoholic extracts of *Griffithsia Pacifica Kylin* were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed by the test compounds in a concentration dependent manner up to the given concentrations in all the models

**DPPH radical scavenging activity:** The hydro alcoholic extract of *Griffithsia Pacifica Kylin* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC<sub>50</sub>) at a concentration of 1.5  $\mu$ g/ml. The result was mentioned in figure 2. The IC<sub>50</sub> value of the extract was found to be lesser than the standard, vitamin C (IC<sub>50</sub> 3.0  $\mu$ g/ml).

**Nitric oxide radical inhibition assay:** The scavenging of nitric oxide by algae extract was increased in a dose-dependent manner as illustrated in Figure 3. At concentration of 116.0  $\mu$ g/ml of extract 50% of nitric oxide generated by incubation was scavenged. This IC<sub>50</sub> value of extract found to be lesser than the standard, rutin (IC<sub>50</sub> 160.0  $\mu$ g/ml).

**Superoxide anion scavenging activity:** The superoxide anion derived from dissolved oxygen by Phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease in absorbance at 560 nm with the plant extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in Figure 4, the algae extract as well as curcumin showed the scavenging activity; IC<sub>50</sub> values, 4.7 µg/ml and 5.84µg/ml, respectively.

**Lipid peroxidation assay:** Activity of algae extract against non-enzymatic lipid peroxidation in rat liver microsomes has been shown in Figure 5. Addition of Fe 2+/ascorbate to the liver microsomes cause increase in lipid peroxidation. The extract showed inhibition of peroxidation effect in all concentrations which showed 50% inhibition effect at 104.0µg/ml. The extract inhibition value was found to be lesser than the standard, vitamin E (IC<sub>50</sub> 120.5µg/ml).

**Hydroxyl radical scavenging assay:** To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H<sub>2</sub>O<sub>2</sub> and ascorbic acid. When the algae extract were incubated with the above reaction mixture, it could prevent the damage against sugar. The results are shown in Figure 6, the concentrations of 50% inhibition were found to be 27.0µg/ml and 32.5 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

**Reducing power:** Figure 7 shows the reductive capabilities of the algae extract compared to butylated hydroxy toluene. The reducing power of extract of *Griffithsia Pacifica Kylin* was very potent and the power of the extract was increased with quantity of sample. The algae extract could reduce the

most Fe<sup>3+</sup> ions, which had a lesser reductive activity than the standard of butylated hydroxy toluene.

**Determination of total phenolic compounds:** The total phenolic contents of hydro alcoholic extract of algae were 0.0589µgpyrocatechol equivalent/mg.

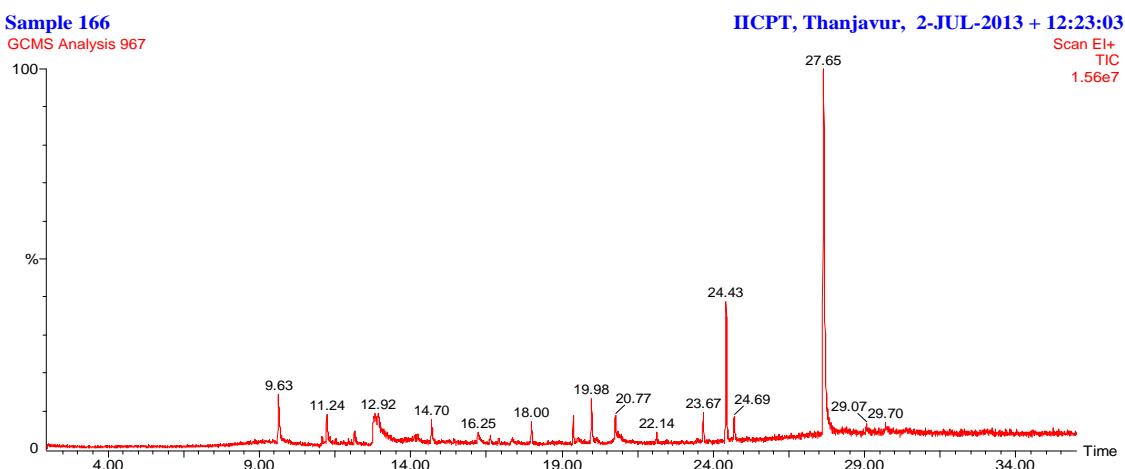
Red marine algae contain pigment phycoerythrin that gives red color to this sea plant, red marine algae as part of Asian dishes and it is especially grown in Japan for commercial, nutritional and medicinal reasons, where it is also dried to be consumed as a snack. The dietary sources of red marine algae contain right blend of nutrients and minerals in the acceptable doses required by the body. Red marine algae are obtained from depths of oceans and serve a variety of functions within human body that includes production of secondary metabolites that aid in detoxification, metabolism and circulation processes.

A lot of experts are in process of devising new medications and pharmacological agents by using active metabolites of red marine algae. Some notable nutritional benefits of red marine algae include provision of essential minerals like calcium, magnesium to meet the standards of recommended daily dosage. Moreover, research data also indicates that red marine algae serves as anti-oxidant to kill/neutralize reactive oxygen species that is preventive against heart diseases and also minimize the risk of stroke in elderly patients. Recent studies revealed that the marine BPs exhibit a wide spectrum of beneficial biological activities (Kim KY, Nam KA, Kurihara H, Kim SM.), and therefore these novel BPs have attracted much attention in the field of functional food and pharmaceutical agents.

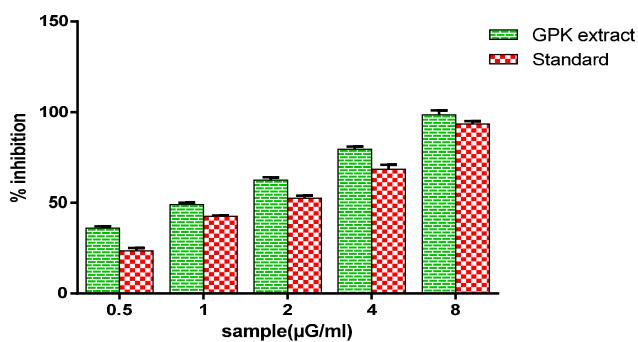
**Table.1 Phytocomponents identified in GPK (GC-MS study)**

	RT	Name of the compound	Molecular formula	MW	Peak Area
1	9.63	1-Iodo-2-methylnonane	C <sub>10</sub> H <sub>21</sub> I	268	7.41
2	11.24	Pentanal, 2,4-dimethyl-	C <sub>7</sub> H <sub>14</sub> O	114	3.10
3	12.15	Decanoic acid, 2-methyl-	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	186	0.96
4	12.92	Nonanoic acid	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	158	5.49
5	14.70	4,8-Dioxaspiro(2.5)oct-1-ene, 6,6-dimethyl-	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	140	2.74
6	16.25	Hexadecanoic acid, 2-oxo-, methyl ester	C <sub>17</sub> H <sub>32</sub> O <sub>3</sub>	284	1.78
7	18.00	2-Bromononane	C <sub>9</sub> H <sub>19</sub> Br	206	1.83
8	19.38	Tridecane, 6-methyl-	C <sub>14</sub> H <sub>30</sub>	198	2.39
9	19.98	Didodecyl phthalate	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	502	4.01
10	22.14	Heptadecane, 9-hexyl-	C <sub>23</sub> H <sub>48</sub>	324	1.02
11	23.67	Trifluoroacetyl-lavandulol	C <sub>12</sub> H <sub>17</sub> F <sub>3</sub> O <sub>2</sub>	250	2.74
12	24.43	Cholesta-4,6-dien-3-ol, (3 $\alpha$ )-	C <sub>27</sub> H <sub>44</sub> O	384	11.58
13	24.69	5 $\alpha$ -Androstan-16-one, cyclic ethylene mercaptole	C <sub>21</sub> H <sub>34</sub> S <sub>2</sub>	350	2.64
14	27.65	Androstan-17-one, 3-ethyl-3-hydroxy-(5-Alpha)	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	318	52.31

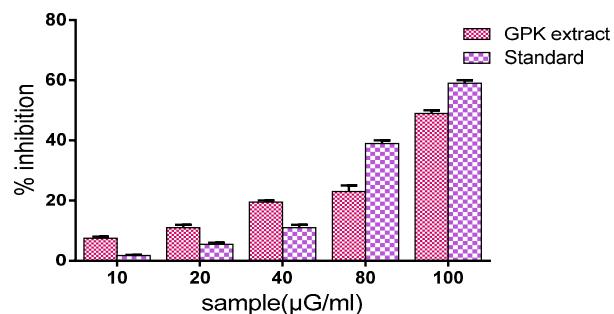
**Fig.1** The chromatogram showing 9.631-iodo -2 –methylnonane, 18.0- 2- Bromononane, 16.2-hexadecanoic acid, 24.43- 5 $\alpha$ -Androstan-16-one, cyclic ethylene mercaptole, 27.65-Androstan-17-one, 3-ethyl-3-hydroxy-(5-Alpha)



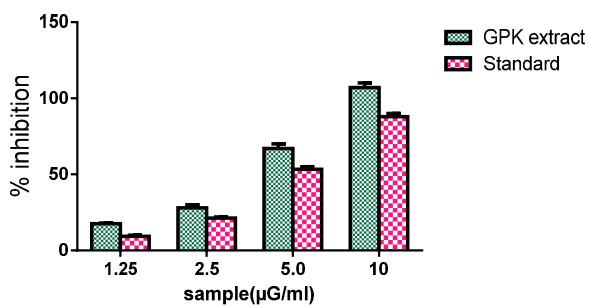
**Fig.2.Scavenging effect of *Griffithsia pacifica kylin* Extract and standard vitamin C on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical. Results are mean  $\pm$  S.D of five parallel measurements**



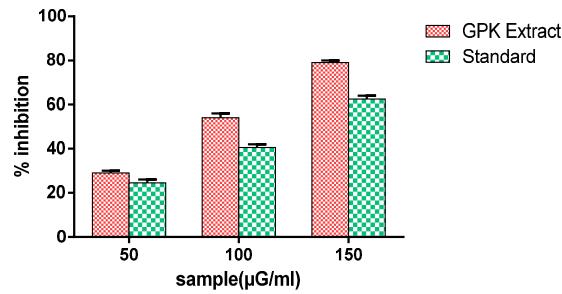
**Fig.3. Scavenging effect of *Griffithsia pacifica kylin* Extract and standard rutin on Nitric oxide radical. Results are mean  $\pm$  S.D of five parallel measurements.**



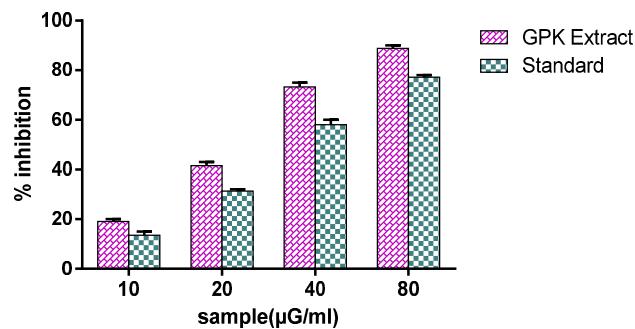
**Fig.4. Effect of *Griffithsia pacifica kylin* Extract and curcumin on scavenging of superoxide anion radical formation. Results are mean  $\pm$  S.D of five parallel measurements.**



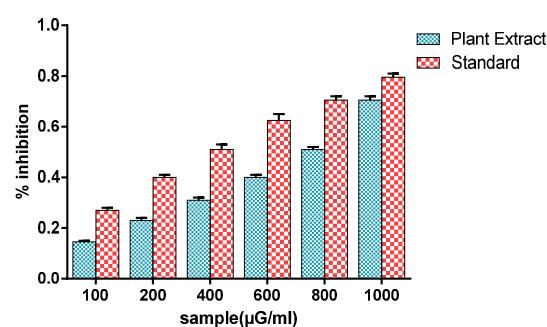
**Fig.5. Effect of *Griffithsia pacifica kylin Extract* and vitamin E on lipid peroxidation of liver microsome induced by Fe<sup>2+</sup>/ascorbate.**  
Results are mean  $\pm$  S.D of five parallel measurements



**Fig.6. Hydroxy radical scavenging activity**  
**Effect of *Griffithsia pacifica kylin Extract* and vitamin E on deoxyribose degradation assay.** Results are mean  $\pm$  S.D of five parallel measurements.



**Fig.7**  
**Reducing power**  
**The reductive ability of *Griffithsia pacifica kylin Extract* and butylated hydroxy toluene.** Results are mean  $\pm$  S.D of five parallel measurements.



Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works.

It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (Rose et al., 1982). Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous algae constituents have proven to show free radical scavenging or antioxidants activity (Aruoma, 1997). Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines etc) of algal origin have been reported as scavengers and inhibitors of lipid peroxidation (Formica, 1995).

In this study demonstrated that, DPPH is a free radical, stable at room temperature, which produces a purple colour solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions. Figure 2 illustrates the decrease in the concentration of DPPH radical due to scavenging ability of hydro alcoholic extract of algae and vitamin C, which is comparable to the reported value of Thabrew et al (Thabrew, 1998). Nitric oxide radical inhibition study proved that the extract is a potent scavenger of nitric oxide. This nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide

compete with oxygen leading to reduced production of nitric oxide (Badami, 2003).

From the nitric oxide test, rutin was used as a standard. The IC<sub>50</sub> value of the rutin is comparable to the reported value of Badami et al (Badami, 2003). In the PMS/NADH - NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Addition of various concentrations of extract as well as curcumin (standard) in above coupling reaction showed decrease in absorbance.

When GPK extract and vitamin E were added to the reaction mixture they removed hydroxyl radicals and prevented the degradation of 2-deoxy-2- ribose as mentioned above. The observed IC<sub>50</sub> values of the extract and Vitamin E were analogous to the reported values of Sen et al (Sen et al., 2002). Figure 7 shows the reductive capabilities of algae extract compared with butylated hydroxy toluene. For the measurements of the reductive ability, we investigated the Fe<sup>3+</sup> to Fe<sup>2+</sup> transformation in the presence of hydro alcoholic extract using the method of Oyaizu et al 1986).

The reducing power increased with increasing the amount of extract. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The absorbance values of the extract at different concentrations were found to be less than that of the reference compound. The value of reference compound is in accordathereport of Illhami et al (Gulcin et al. 2002). The phenolic compounds may contribute directly to anti oxidative action (Duh et al., 1999). This result indicates that

hydrocarbons and alkanes present in algae extract could be partly responsible for the beneficial effects. Compelling evidence indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging.

This study suggested that the *Griffithsia Pacifica Kylin* extract possess strong free radical scavenging, lipid peroxidation property and could be explored as natural antioxidant, that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. The antioxidant activity due to the presence of phyto compounds like Hexadecanoic acid, Nonanoic acid, Heptadecane,9-hexyl-which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the *Griffithsia Pacifica Kylin* lead to chemical entities with potential for clinical use and bioavailability.

#### *Griffithsia pacifica Kylin* Photograph



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