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# Antioxidant activities of a few common seaweeds from Gulf of Mannar and the effect of drying as the method of preservation

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**Abstract :** *The pharmaceutical, food and several other industries have experienced great expansion in the demand for seaweeds due to their significant applications as ingredients in functional foods and richness in antioxidant ingredients. The three selected seaweeds found in the coastal areas of Gulf of Mannar, Tamil Nadu are Sargassum wightii, Gracilaria corticata and Kappaphycus alvarezii. These samples were investigated for antioxidant activity due to the presence of phytochemicals like tannins and polyphenols. Commercially, many marine algae are used in the production of various food additives. In such case, the formulation of products from algae involves drying as a major step in preservation. Thus, the effect of different drying temperatures on the phytochemical constituents in seaweed after harvest is evaluated. The potential difference between the fresh and dried seaweeds as a possible food supplement is discussed with the data. Further, the antioxidant activity after extraction of essential products like carrageen, agar and alginate is studied and the impact of drying on the same.*

***Key Words***: *Sargassum wightii*, *Gracilaria corticata*, *Kappaphycus alvarezii*, phytochemicals, antioxidant activity

## 1. INTRODUCTION

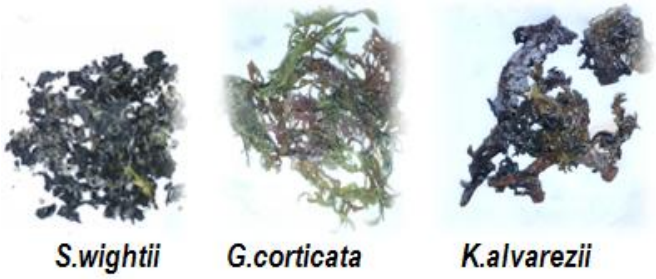
Bio-stimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anticoagulants and suspending agents in radiological preparations. Seaweeds have recently received significant attention for their potential as natural antioxidants. Fresh and dried seaweeds are utilized as human food ([13] K. Nisizawa et al., 2002) especially in coastal areas; the consumption of seaweed is high when compared to other areas ([10] N. Kaliaperumal et al., 1995). Dietary seaweeds include marine algae from brown, green and red taxonomies such as the Laminariales, Ulvales and Porphyridiales, respectively ([2] Anggadiredja et al., 1997; [21] Yan et al., 1998). Lipid peroxidation leads to deterioration of biological systems due to reactive oxygen species. Butylated hydroxytoluene and butylated hydroxyanisole have been suspected of being responsible for liver damage and carcinogenesis ([3] Grice, 1986; [19] Wichi, 1988; [5] Hettiarachchy et al., 1996). Antioxidant activity of marine algae may arise from carotenoids, tocopherols and polyphenols. These compounds directly or indirectly contribute to inhibition or suppression of free radical generation ([1] Y. Athukorala et al., 2003). Air drying is the most frequently used dehydration operation in the food and chemical industry ([7] Ibrahim et al., 2009). The wide variety of seaweeds, available to the consumers and the interesting concern for meeting quality specifications and energy conservation, emphasize the need for a thorough understanding of the drying process. For example Red seaweed Porphyra or commonly called as Nori or Laver is dried and processed into thin purplish black sheets according to FAO document ([22] Dennis J. McHugh 2003). The extent of drying process may affect the quality in terms of antioxidant or free radical scavenging activity is not much known. Hence, the present study focus upon antioxidant activity of selected three seaweeds in fresh and dry conditions. Further, the antioxidant activity after extraction of essential products like carrageen, agar and alginate is studied and the impact of drying on the same.

## 2. MATERIALS AND METHODS

### 2.1. Collection and Extraction of seaweeds

The selected seaweeds - *Sargassum wightii*, *Gracilaria corticata* and *Kappaphycus alvarezii* were collected from Gulf of Mannar, Tamil Nadu. The samples were washed thoroughly in seawater followed by tap water and finally in distilled water to remove unwanted salts and other materials. 10g of each seaweed were weighed in which 5 g was shade dried and other 5 g was sun dried. Aqueous extractions of all the three seaweeds were performed by adding 1 g of seaweed to 6 mL of phosphate buffer and incubated for 24 hours. The supernatant was carefully separated and kept in airtight amber bottle and stored for conducting different assays.

**Fig 1:** Three different seaweeds




### 2.2 Extraction of carrageenan and alginate

*Kappaphycus alvarezii* a carragenophyte was used in the extraction. Carrageenan extraction was performed as described previously ([15] Ohno et al., 1994) with slight modifications. The alginate extraction was performed using *Sargassum wightii* according to [14] Nishigawa (1985).

### 2.3 Determination of total phenolic content

Total phenolic content assay in each extracts were measured using Folin-Ciocalteu method ([20] Kahkonen *et al.*, 1999). 5 mL of Folin-Ciocalteu phenol reagent and 4 mL of 7.5% (w/v) sodium carbonate were added to 1 mL of seaweed extracts which were incubated for 2 hours in a dark place at room temperature for the reaction to take place. The absorbance of the reaction mixtures were measured at 765 nm wavelength using spectrophotometer. Gallic acid standard was used for comparative study.

### 2.4 Nitric oxide scavenging assay

Phenolic compounds are considered to exhibit radical scavenging properties ([18] Umayaparvathi S et al., 2012). The nitric oxide scavenging assay was performed by adding 1.5 mL of each extract to separate test tubes (1.5 mL distilled water used as control instead of extract) to which 1.5 mL of 10 mM sodium nitrite was added and incubated for 2 hours 30 minutes. After incubation 1 mL of incubated samples were added with 2 mL of 0.33% sulphanilic acid and left for another incubation period of 5 minutes at room temperature. Freshly prepared 300µl of 0.1% napthol was added and the absorbance was measured at 540 nm after incubation period.

### 2.5 Assay for inhibition of lipid peroxidation

An oleic acid emulsion was prepared by adding 0.25 mL of oleic acid with 9.75 mL of isopropanol. 0.5 mL of extract was added with 0.5 mL of isopropanol and 1 mL of 0.05M phosphate buffer and stored in dark. At regular intervals of time (0 and 24 hours), 0.1 mL of incubated sample was taken for the measurement of degree of oxidation by ferric thiocyanate method. The absorbance was recorded at 500 nm.

### 2.6 Beta-carotene bleaching(BCB) assay

β -carotene bleaching assay was performed as described by ([9] T. Juntachote and E. Berghofer, 2005). Aliquot 3 ml of the β-carotene emulsion was added to 40 mg of linoleic acid and 400 mg of Tween 40. After the chloroform has evaporated, 100 mL of distilled water was added to the mixture and mixed vigorously. The initial absorbance was recorded at 470 nm and 700 nm immediately. From which 3 mL of β-carotene/linoleic acid emulsion were mixed with 100 µL of each seaweed extract. The tubes were incubated at 50°C for 60minutes and absorbance was recorded at 470 nm and 700 nm. Degradation rate (DR) of β-carotene = [ln (A initial /A sample)]/60

Antioxidant activity (%) = [(DRcontrol – DRsample)/DRcontrol] x 100

### 2.7 Ferrous ion chelating (FIC) activity

The ferric ion chelating assay was conducted using ferrous sulphate and ferrozine ([16] N. Singh and P.S. Rajini, 2004). 0.1 mL of extract solution was added to 1.0 ml of 0.1mM FeSO4 and 1.0 ml of 0.25mM ferrozine. The tubes were shaken well and left undisturbed for 10 mins. The absorbance was measured at 562 nm. Blank was prepared by replacing ferrozine with water whereas the control consisted of water in place of the extract.

Chelating effect (%) = (Acontrol- Asample)/Acontrol x 100

### 2.8 Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was performed with slight modifications from ([6] HowYee Lai and YauYan Lim, 2011). 1 mL of each extract was added to 2.5 mL of 0.2M phosphate buffer (pH 6) and 2.5 mL of potassium ferricyanide (1% w/v) and incubated for 20 min at 50°C, after which 2.5 mL of 10% trichloroacetic acid was added. An aliquot of 2.5 mL of each mixture was diluted, before adding 0.5 mL of (0.1% w/v) ferric chloride and incubated for about 30 minutes. Absorbance was measured at 700 nm. A calibration curve was constructed using gallic acid.

## 3. RESULTS AND DISCUSSION

### 3.1. Total phenolic content

A number of studies have focussed on the biological activities of phenolic compounds, which are potential antioxidants and free radical-scavengers ([17] Sugihara et al., 1999). The phenol content of *Kappaphycus alvarezii* was found higher than others. Sargassum wightii had quite lower phenolic content than *K.alvarezii* but much higher than *G.corticata*. After carrageen extraction and alginate extraction, the phenolic content was reduced. Similarly, in case of drying both shade drying and sun drying reduced the total phenolic content. It is interesting to see that the extraction of carrageen reduced the phenolic content to almost half its original value in *K. alvarezii*, whereas, the phenolic content was not greatly affected by the extraction of alginate in the case of *S.wightii*.

Sun drying affected the phenolic content in the case of *G. corticata* more than that of shade dried one, though reason could not be explained.

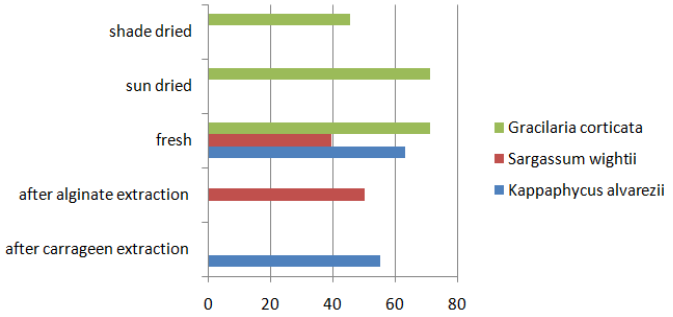
**Table 1**: Total phenolic content of seaweed extracts

| **Seaweed extracts** | **Absorbance** 765nm | **Total phenolic content** (mg (GAE)/100gdw seaweed) |
|---|---|---|
| Kappaphycus alvarezii | 0.96 | 0.92 |
| K. alvarezii (after carrageen extraction) | 0.63 | 0.58 |
| Sargassum wightii | 0.77 | 0.61 |
| S. wightii (after alginate extraction) | 0.61 | 0.54 |
| Gracilaria corticata (fresh) | 0.34 | 0.27 |
| G. corticata (shade dried) | 0.27 | 0.20 |
| G. corticata (sun dried) | 0.19 | 0.15 |

### 3.2. Nitric oxide scavenging assay

The scavenging effect of the seaweeds measured by this assay resulted in proving that *G.corticata* has the largest scavenging activity compared to others, but the activity of *K.alvarezii* was higher than that of *S.wightii*. Similar to total phenolic content, Nitric oxide scavenging effect was not much affected by alginate extraction procedure in *S.wightii*, except that there was a slight increase in the activity. The reduction of Nitric oxide scavenging effect of *K. alvarezii* was relatively higher when compared to the phenolic content.

**Fig 2:** Nitric oxide scavenging effect of seaweed extracts



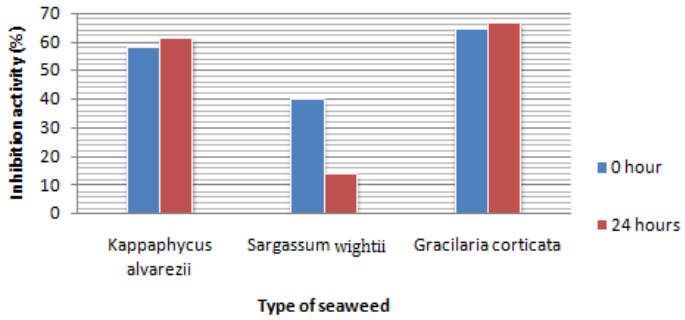

### 3.3. Assay for inhibition of lipid peroxidation

Inhibition activity of *G.corticata* and *K.alvarezii* was found to be higher than other extract - before and after incubation. Further, this activity was slightly increased on incubation in *G.corticata* and *K.alvarezii* whereas in *S.wightii* the inhibition was reduced after 24 hours. This reduction in the inhibition of lipid peroxidation in the case of *S.wightii* could possibly be attributed to compounds that are either volatile or get destroyed due to oxidation with incubation.

**Table 3**: Lipid peroxidation - Inhibition assay

| Seaweed extracts | Absorbance | | Inhibiting activity (%) | |
|---|---|---|---|---|
| | 0 hour | 24 hours | 0 hour | 24 hours |
| Kappaphycus alvarezii | 0.19 | 0.14 | 57.77 | 61.11 |
| Sargassum wightii | 0.27 | 0.31 | 40.00 | 13.88 |
| Gracilaria corticata (fresh) | 0.16 | 0.12 | 64.44 | 66.66 |
| Control | 0.45 | 0.36 | - | - |

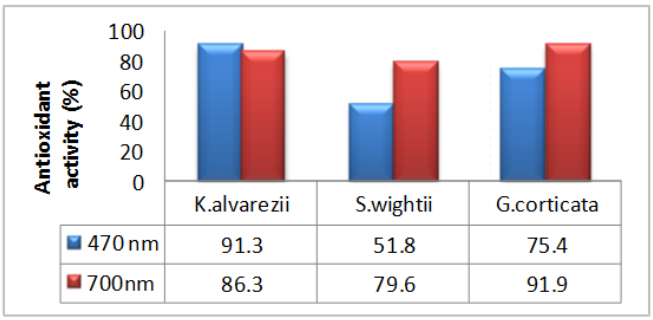
**Fig 3:** Lipid peroxidation - Inhibition assay




### 3.4. Beta-carotene bleaching assay

BCB method measured the ability of an antioxidant to inhibit lipid peroxidation. In the BCB method, a model system made of -carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The antioxidant activity was expressed as percent inhibition relative to the control. In general there was an appreciable inhibition of beta carotene bleaching activity with all the 3 seaweeds studied.

**Fig 4:** Beta carotene bleaching - inhibition assay




### 3.5. Ferrous ion chelating (FIC) activity

$Fe^{2+}$ has been known to accelerate formation of hydroxyl radicals via the Fenton reaction, leading to occurrence of many diseases ([4] B. Halliwell., 1996). It is reported that chelating agents that form bonds with a metal ion, are effective secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion ([11] K.S. Kumar et al., 2008). Ferrozine can quantitatively chelate with $Fe^{2+}$ and form a red coloured complex. The chelating activity of *K.alvarezii* reduced after carrageen extraction whereas it increased almost twice in the case of *S.wightii*. The activity of shade dried *G.corticata* increased about 40% compared to fresh seaweed whereas sun drying has resulted in only a marginal increase in activity. It is possible that sun drying due to photo bleaching and higher temperature can destroy some of the compounds that were not affected by shade drying.

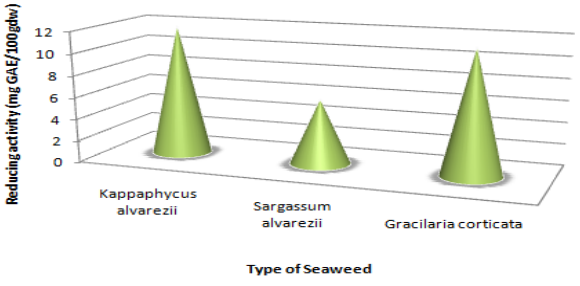
**Table 4:** Ferrous ion chelating activity

| Seaweed extracts | Absorbance 562nm | Chelating activity (%) |
|---|---|---|
| Kappaphycus alvarezii | 0.39 | 41.79 |
| K. alvarezii (after carrageen extraction) | 0.52 | 22.38 |
| Sargassum wightii | 0.56 | 16.41 |
| S. wightii (after alginate extraction) | 0.48 | 28.35 |
| Gracilaria corticata (fresh) | 0.38 | 43.28 |
| G. corticata (shade dried) | 0.26 | 61.19 |
| G. corticata (sun dried) | 0.34 | 49.25 |
| Control | 0.67 | - |

### 3.6. Ferric ion reducing antioxidant power (FRAP) assay

In this assay, the ferric ions are reduced to ferrous, which is a redox reaction involving single electron transfer by the action of antioxidants in the seaweed extracts which directly signifies the reducing power. Our present study has shown that *S.wightii* has got lowest reducing activity compared to *K.alvarezii* and *G.corticata* which showed almost equal ferric ion reducing activity which may be attributed by the difference in reactivity due to compounds that are responsible for its reducing potential.

**Fig 4:** Ferric ion reducing activity




## 4. CONCLUSIONS

The difference in the correlations between TPC and antioxidant assays indicates the versatility of the group of phenolic compounds in the seaweeds and their different responses to different methods for the determination of the antioxidant activity. This difference is due to the fact that Folin-Ciocalteau method determined the sum of phenolic compounds, whereas individual phenolic compounds have very different responses on the Folin Ciocalteau reagent and antioxidant activity ([12] B.Matthaus, 2005). The difference found in various activities within the same seaweed or among different seaweeds in various activities could be attributed due the presence of various phytochemicals present in them. More studies on these molecules and their activity, stability and their usefulness as nutraceuticals in human health need to be studied thoroughly. Sun drying is widely used for processing and was found to increase the chelating activity and phenolic content but shade drying does not produce much effect on the phytochemicals. In general there are some reductions in the quantity of total phenolic content after carrageenan extraction which also reflects in its antioxidant and free radical scavenging activity. But in the case of alginic acid extraction from *Sargassum wightii*, there is some increase in the total phenolic activity as well as antioxidant and free radical scavenging activity. It could be due to the fact the process involved in carrageenan production might remove part of the polyphenols and in the alginate process, they might get concentrated or the active molecules involved could be contributed by substances other than polyphenols as we can see that in fact there are some reductions in polyphenols after alginate extraction. Retention of activity to a greater extent gives a hope of using the waste from seaweed industry for obtaining value added compounds from seaweeds.

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## REFERENCES

[1] Y. Athukorala, K.W. Lee, S. Choonbok, B.A. Chang, S.S. Tai, Potential antioxidant activity of marine red algae (*Grateloupia filicina*), Journal of Food Lipid 10 (2003) 251-265.

[2] Anggadiredja, J., Andyani, R., & Hayati, M. (1997). Antioxidant activity of Sargassum polycystum (Phaeophyta) and Laurencia obtusa (Rhodophyta) from Seribu islands. Journal of Applied Phycology, 9, 477–479.

[3] Grice H.C. (1986). Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food and Chemical Toxicology 24:1127–1130.

[4] B. Halliwell, "Antioxidants in human health and disease," *Annual.Review of Nutrition*, vol. 6, pp. 33-50, July 1996.

[5] Hettiarachchy N.S., Glenn K.C., Gnanasambandam R. and Johnson M.G.. (1996). Natural antioxidant extract from fenugreek (Trigonella foenumgraecum) for ground beef patties. Journal Food Science 61: 516–519.

[6] HowYee Lai and YauYan Lim, "Evaluation of Antioxidant Activities of the Methanolic Extracts of Selected Ferns in Malaysia," International Journal of Environmental Science and Development, Vol. 2, No. 6, December 2011.

[7] Ibrahim, M., K. Sopian and W.R.W. Daud. 2009. Study of the drying kinetics of lemon grass. American Journal 0f Applied Sciences 6 (6): 1070-1075.

[8] Je JY,et al.Antioxidant activity of enzymatic extracts from the brown seaweed Undaria pinnatifida by electron spin resonance spectroscopy. LWT-Food Sci Technol 2009; 42: 874-878.

[9] T. Juntachote and E. Berghofer, "Antioxidative, properties and stability of ethanolic extracts of Holy Basil and Galangal," Food Chemistry, vol. 92, pp. 193-202. Sept. 2005.

[10] N. Kaliaperumal, S. Kalimuthu, J.R. Ramalingam, Economically Important Seaweeds, CMFRI-Special Publication, Cochin, 1995, pp. 40-62.

[11] K.S. Kumar, K. Ganesan, P.V.S. Rao, "Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) – An edible seaweed," *Food Chemistry*, vol. 107, pp. 289-295, March 2008.

[12] B.Matthaus, "Antioxidant activity of extracts obtained from residues of different oilseed," *Journal of Agricultural and Food Chemistry*, vol. 50, pp. 3444-3452, May 2002.

[13] K. Nisizawa, Kaiso, Bountiful Harvest from the Sea's sustenance for Health and Well Being by Preventing Common Life Style Diseases, Japan Seaweed Association, Japan, 2002, pp. 59-6

[14] Nishigawa, K., 1985. .Fr.xtract method of alginic acid. In, Research nzethod of algae, edited by K. Nishigawa and M. Chthara, pp. 624-626.

[15] Ohno, M, D.B. LARGo and T.IKuMo'ro, 1994. Growth rate, carrageenan yield and gel properties of cultured kappa carrageenan producing red alga KaPPmphyeus alvarezii CDoty) Doty in the subtropical waters of Shikoku, Japan. Apt)t Phycology, 6. pp. 1-5.

[16] N. Singh, P.S. Rajini, "Free scavenging activity of an aqueous extract of potato peel," Food Chemistry, vol. 85, pp. 611-616, May 2004.

[17] Sugihara, N., Arakawa, T., Ohnishi, M., & Furuno, K. Anti and pro-oxidative effects of flavonoids on metal induced lipid hydroperoxide- dependent lipid peroxidation in cultured hepatocytes located with /-linolenic acid. Free Radical Biology and Medicine, 27, 1313–1323, 1999.

[18] Umayaparvathi S, Arumugam M, Balasubramanian T. Meenakshi S. *In vitro* antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. Asian Pac J Trop Biomed.2012;1(Suppl 1):S66–S70.

[19] Wichi H.P. (1988). Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal aquamous epithelium. Food and Chemical Toxicology 26: 717–723.

[20] M.P. Kahkonen, A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala, M. Heinonen. "Antioxidant activity of plant extracts containing phenolic compounds," *Journal of Agricutural and Food Chemistry*, vol. 47, pp. 3954-3962, February 1999.

[21] Yan, X., Nagata, T., & Fan, X. (1998). Antioxidative activities in some common seaweeds. Plant Foods for Human Nutrition, 52, 253–262.

[22] Dennis J. McHugh (2003) A guide to the Seweed Industry in FAO Fisheries Technical Paper, FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS