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# Biochemical composition, alginic acid yield and antioxidant activity of brown seaweeds from Mandapam region, Gulf of Mannar

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ABSTRACT

seaweeds.

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Kev words: Bioactive compounds, antioxidant activity, DPPH, alginic acid yield, viscosity.

## **INTRODUCTION**

The marine environment in which seaweed exist possess great taxonomic diversity and synthesise metabolites with varied structure with interesting biological activities for food material and medical applications (Batista et al., 2009). Antioxidant, dietary fibre, essential fatty acids, vitamins and minerals are rich source of bioactive compounds obtained from seaweeds (Draw - Vrillon, 1983; Chandini et al., 2008). Extraction of seaweeds shows strong antioxidant activity (Barrow & Shahidi, 2008; Gamal- Eldeen et al., 2009). The Phaeophyta (brown seaweeds) shows comparatively higher antioxidant activity than green and red algae (Al-Amoudi, 2012). Seaweeds contain different variety of inorganic and organic substances which can be used for human health for examples polyphenols, carotenoids and tocopherols, terpenes, ascorbic acid, alkoloid (Chanda et al., 2010). This addition of compounds have demonstrated antioxidant activity in a variety of in vitro studies (Heo et al., 2009).

Free radicals are produced as a part of normal metabolic processes. Reactive oxygen species (ROS) include free radical for example, hydroxyl radical(OH), superoxide anion (O<sub>2</sub>) and

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triquetra. The viscosity of sodium alginate was found to be high in 5 hours of extraction period in all the non free radical species such as hydrogen peroxide  $(H_2O_2)$ , nitric oxide (NO) are various forms of activated oxygen and are destructive to various physiologically important molecules including protein, lipids, cell membrane, DNA and other cellular constituents (Wijeratne et al., 2005). It induces different types of

In this present study, four brown seaweeds viz Sargassum wightii Greville, Padina tetrastromatica Hauck,

Chnoospora minima, Hormophysa triquetra (C. Ag.) Kutz, collected from Mandapam region of Gulf of Mannar

were analyzed for its bioactive potentials. High protein content was observed in Hormophysa triquetra

(15.34±0.01%) and carbohydrate (59.30±0.66%); lipid (0.55±0.002%) were recorded in Padina tetrastromatica.

Total phenolic content (20±3.46 mg GAE /g) was found to be high in Sargassum wightii, whereas antioxidant activity (34.66±5.77 mg AAE /g) in Padina tetrastromatica. Flavonoid content (66.3±1.43 mg QE /g), DPPH

radical scavenging activity (85.08±1.17%) and alginic acid yield (26.70%) was found to be high in Hormophysa

serious human diseases such as atherosclerosis, rheumatoid arthritis muscular dystrophy, cataracts, some neurological disorders and some type of cancer as well as aging (Kovatcheva et al., 2001). Sodium benzoate, sodium nitrite are synthetic antimicrobials and butylated hydroxyanisole, butylated hydroxytoluene, tert-butyl hydroxyquinone are synthetic antioxidant. It is commonly used in food industry for preserving food and its quality but those have been suspected of toxic and exerting carcinogenic effect (Gupta & Abu-Ghannam, 2011).

Alginate forms a major structural polysaccharide of many marine brown algae comprising up to 40% of the dry matter (Haug, 1964). It is a family of linear  $(1\rightarrow 4)$ -linked  $\alpha$ -L-gulurono  $\beta$ -Dmannuronans of widely varying composition and sequential structure (Painter, 1983). Seaweeds are used as food in many countries like China, Japan and Taiwan. In India, their use as food is very limited (Thinakaran & Sivakumar, 2012). Alginate is widely used in textile printing, paper coating and other relatively low margin industrial applications. The only other derivative of alginic acid that is used in the food industry is propylene glycol

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alginate or PGA (Bixler & Porse, 2010). There is a large potentials for alginate in biotechnological applications. Alginate is widely used in industry because of its ability to retain water, and its gelling, viscosifying and stabilising properties (Haug, 1964). So the aim of the present study is to analyze the bioactive compounds from four algin yielding seaweeds *viz, Sargassum wightii Greville, Padina tetrastromatica Hauck, Chnoospora minima,* and *Hormophysa triquetra* (C.Ag.) Kutz, collected from Mandapam region of Gulf of Mannar.

## MATERIALS AND METHODS

## Sample collection and preparation

Sargassum wightii Greville, Padina tetrastromatica Hauck, Chnoospora minima, and Hormophysa triquetra (C. Ag.) Kutz, plants were collected from Mandapam region of Gulf of Mannar. The algae were washed with tap water to remove dirt, sand and then the same was shade dried until constant weight is obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

## **Extraction procedure**

0.5 g of seaweed powder was extracted with 10 ml of 80% methanol at 35°C in a shaking water bath. After 24 h the samples were cooled down to the room temperature and centrifuged at 4000 rpm for 10 min. The supernatant was recovered for the antioxidant activity (Cai *et al.*, 2004).

## **Biochemical composition**

# Protein estimation

The total protein was estimated using the Lowry method (1951). The protein was calculated by using BSA as a standard and expressed in percentage.

#### Carbohydrate estimation

The total carbohydrate was estimated by following the phenol-sulphuric acid method of Dubois *et al.*, (1956). The carbohydrate content was calculated by referring to a standard D-glucose and the results are expressed in percentage.

## Lipid estimation

The extraction of lipid was done by the chloroformmethanol mixture by using Folch *et al.*,(1956) and it is expressed in percentage.

# Total phenolic content

The amount of total phenolics in methanol extract was determined with Folin– Ciocalteu reagent according to the method of Singleton and Rossi (1965) with Gallic acid as the standard. Briefly standard stock solution of 10 mg/10 ml of gallic acid was prepared in distilled water. From this, various concentrations ranging from 200-1000  $\mu$ g/ml were prepared. To this 1 ml Folin and Ciacalteau reagents (1:2 with

water) was added and kept at room temperature for 5 min and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The colour developed was read at 750 nm. A 100  $\mu$ l of methanol extract of sample was mixed with the same reagents. Gallic acid was used as the reference standard and the results are expressed as milligram gallic acid equivalent (mg / g dry weight of seaweed material. All samples were analysed in triplicate.

# Total antioxidant activity

Total antioxidant activity of seaweed extracts was determined according to the method of Prieto *et al.*, (1999). Briefly 0.3 ml of sample was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then reaction mixture was incubated at  $95^{\circ}$  C for 90 min. Absorbance of all the sample was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid in milligram per gram of extract.

## Total flavonoid content

Aluminium chloride colorimetric technique was used for flavonoids estimation (Chang *et al.*, 2002). 0.5 ml of sample, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, 2.8 ml of distilled water were taken and mixed in the given order. It was left at room temperature for 30 minutes after which the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared using Quercetin solution (20-100µg) in methanol.

#### **DPPH** radical - scavenging activity

The scavenging effects of samples for DPPH radical were monitored according to the method of Yen and Chen (1995) Briefly, 2.0 ml of aliquot of test sample was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexes for 1 minutes and then left to stand at room temperature for 30 minutes in the dark, and its absorbance was read at 517 nm. The percentage of scavenge the DPPH radical was calculated using the following equation. Gallic acid was used as positive controls.

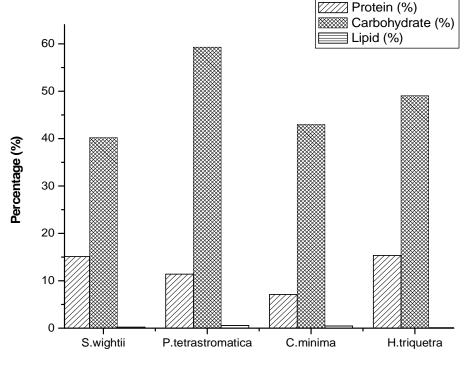
DPPH scavenging activity (%) =  $[(Ac-As)/Ac] \times 100$ 

where Ac is the absorbance of the control  $(100\mu L$  of ethanol with  $100\mu L~$  of the DPPH solution ) and As is the absorbance of the sample.

#### Extraction of alginic acid

The Suzuki method (1955) was adopted to extract alginic acid. The extraction was monitored for 1hr and 5hr at 80° C. The precipitate obtained was dried under ambient conditions. The crude alginic acid extracted was converted into sodium alginate by adding 5% sodium carbonate solution. The viscosity of 1% alginate samples was determined using Ostwald's viscometer. The values were expressed in mPa.

Sl.No Name of the brown seaweeds <u>1 hour</u> Yield of alginic acid(%) Viscosity (mPa) Yield of alginic acid	5 hour
Yield of alginic acid(%) Viscosity (mPa) Yield of alginic acid	
	d(%) Viscosity (mPa)
1 Sargassum wightii 14.21% 12.82 mPa 21.71%	14.32 mPa
2 <i>Padina tetrastromatica</i> 12.40% 12.64 mPa 19.70%	14.14 mPa
3 <i>Chnoospora minima</i> 12.70% 11.99 mPa 20.20%	13.49 mPa
4 <i>Hormophysa triquetra</i> 19.20% 13.59 mpa 26.70%	15.09 mPa



#### **Biochemical composition**

Fig. 1: Biochemical composition of brown seaweeds collected from Mandapam region of Gulf of Mannar.

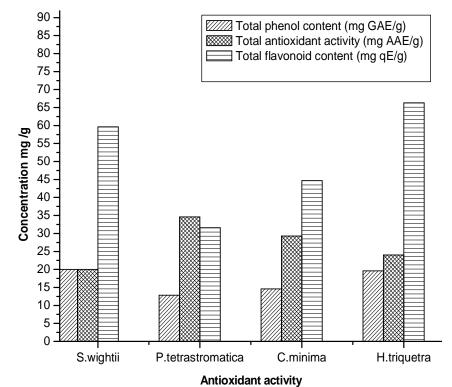


Fig. 2: Antioxidant activity of brown seaweeds collected from Mandapam region of Gulf of Mannar.

## RESULTS

## **Biochemical composition**

In the present investigation total protein content varied from 7.11 $\pm$ 0.01 to 15.34 $\pm$ 0.01%. The high protein content was recorded in *Hormophysa triquetra* (15.34 $\pm$ 0.01%) followed by *Sargassum wightii* (15.10 $\pm$ 0.08%), *Padina tetrastromatica* (11.39 $\pm$ 0.02%), while low protein content was observed in *Chnoospora minima* (7.11 $\pm$ 0.01%).

The protein values are significantly different between the four seaweeds (P < 0.05). The maximum carbohydrate was recorded in *Padina tetrastromatica* (59.30 $\pm$ 0.66%) followed by *Hormophysa triquetra* (49.06 $\pm$ 1.02%), *Chnoospora minima* (42.98 $\pm$ 1.12%) while minimum carbohydrate content was observed in *Sargassum wightii* (40.21 $\pm$ 0.66%).

The carbohydrate values are significantly different between the four seaweeds (P < 0.05). The lipid content varied from (0.55 $\pm$ 0.002% to 0.11 $\pm$ 0.001%). High lipid content was recorded in *Padina tetrastromatica* (0.55 $\pm$ 0.002%), followed by *Chnoospora minima*,(0.45 $\pm$ 0.002%), *Sargassum wightii*,(0.21 $\pm$ 0.001%), while low lipid content was observed in *Hormophysa triquetra* (0.11 $\pm$ 0.001%). The lipid values are significantly different between the four seaweeds (P < 0.05). Figure.1

# **Total phenol content**

The maximum phenol content was observed in *Sargassum wightii* (20 $\pm$ 3.46 mg /g) followed by *Hormophysa triquetra* (19.6 $\pm$ 1.52 mg /g), *Chnoospora minima* (14.66 $\pm$ 1.15mg /g), while the minimum phenol content was observed in *Padina tetrastromatica* (12.83 $\pm$ 1.04 mg /g). The total phenol content of methanolic extracts are significantly different between the four seaweeds (P < 0.05). Figure.2

# Total antioxidant activity

The total antioxidant activity of methanol extract of four different brown seaweeds are presented in Fig 2. Padina showed highest total antioxidant tetrastromatica activity  $(34.66 \pm 5.77)$ ascorbic acid equivalent/g) mg followed by Chnoospora minima (29.3±9.86 mg/g), Hormophysa triquetra (24.0±3.05 mg /g) and Sargassum wightii showed lowest antioxidant activity (20.0±2 mg /g). The total antioxidant activity of methanolic extracts are significantly different between the four seaweeds (P <0.05).

## **Total Flavonoid content**

Hormophysa triquetra was found contain to flavonoid ( $66.3\pm1.43$  mg quercetin equvalent/g) maximum wightii followed by Sargassum (59.66±1.52mg /g), Chnoospora minima (44.7±1.25mg /g) and minimum content was observed in Padina tetrastromatica (31.60±4.04 mg /g). The total flavonoid content of methanolic extracts are significantly different between the four seaweeds (P < 0.05). Fig 2

#### **DPPH** free radical scavenging activity

The free radical scavenging activity of methanolic extract of seaweeds was studied. The maximum activity was found in *Hormophysa triquetra* ( $85.08\pm1.17\%$ ), followed by *Sargassum wightii* ( $69.31\pm0.70\%$ ), *Padina tetrastromatica* ( $61.04\pm0.93\%$ ) and minimum free radical scavenging activity was observed in *Chnoospora minima* ( $46.91\pm1.32\%$ ). The DPPH free radical scavenging activity of methanolic extracts are significantly different between the four seaweeds (P < 0.05).

# Alginic acid yield and viscosity

The total alginate yield obtained from *Sargassum wightii*, *Padina tetrastromatica, Chnoospora minima* and *Hormophysa triquetra* are shown in Table .1 .The maximum alginic acid yield was obtained from *Hormophysa triquetra* (26.70%) and lowest yield was obtained from *Padina tetrastromatica* (19.70%). The alginic acid yield are significantly different between the four seaweeds (P < 0.05). High viscosity was observed in *Hormophysa triquetra* (15.09 mPa) and minimum was observed in *Chnoospora minima* (13.49 mPa) after 5 hours of extraction period.

# DISCUSSION

Studies on the chemical composition of seaweeds have shown that these are good sources of proteins, lipids carbohydrates minerals and trace elements. The protein content in brown seaweeds are generally lower ranging from 5 to 15% of dry weight of seaweed (Burtin, 2003; Chakraborty & Santra, 2008; Manivannan *et al.*, 2008, 2009; Rohani-Ghadikolaei *et al.*, 2011).In the present study all the four species of brown algae showed more or less similar values for protein. It has been observed that the protein content of seaweed is dependent on season and environmental growth conditions (Dawczynski *et al.*, 2007). Besides the low protein content, it has been shown that these seaweeds are rich in essential amino acids (Joel Fleurence, 1999).

Carbohydrate is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. The typical carbohydrates in brown seaweeds are fucoidan, laminaran, cellulose and alginates (Dawczynski *et al.*, 2007). According to the study done by Marinho-Soriano *et al* (2006) the synthesis of carbohydrates seemed to be favoured by both, intensity of light and temperature while decreasing the proteins and lipids content. Similarly in the present study also the carbohydrate content was high when compared to other species (Chennubhotia *et al.*, 1987; Rameshkumar *et al.*, 2012; Murugaiyan & Narasimman, 2012; Anantharaman *et al.*, 2013; Goecke *et al.*, 2012).

Although macroalgae have been reported to have low lipid contents (Mabeau & Fleurence, 1993), their polyunsaturated fatty acid (PUFA) composition is superior to those of terrestrial vegetables in regard to the human diet (Kumari *et al.*, 2010). The lipid content of seaweeds reported from Mandapam region varied from 3.15% to 5.30% (Anantharaman *et al.*, 2013). The levels of

lipids detected in the present study were lower than previously reported for other brown seaweed species (Seenivasan et al., 2012). Different types of antioxidants in brown seaweeds have been reported (Chandini et al., 2008; Yan et al., 1996; Yan et al., 1999; Ngo et al., 2011; Gupta and Abu-Ghannam, 2011). Phenolics play a primary role as structural components of cell walls and may have secondary roles in signalling, defence (Amsler & Fairhead, 2006)or in responses to environmental stress. In the present study the total flavonoid content and total antioxidant activity and DPPH assay of methanolic extract of S.wightii was found higher than earlier reported by Meenakshi et al.,(2009). The DPPH radical scavenging activity of Hormophysa extract showed significantly higher activity followed by Sargassum wightii (69.31%) P. tetrastomatica (61.04%) and Choonospora (46.91%) than earlier reports (Chandini et al., 2008; Ganesan, Kumar, & Bhaskar, 2008). The present results indicates that all the four species could be an important source of antioxidant molecules.

The alginates are widely utilized as gelling agents in pharmaceutical and food applications. The role of alginate in human health has broadened with the recognition that they have a number of potentially beneficial physiological effects in the gastrointestinal tract. The role of appropriately designed alginate formulations in the management of overweight and obesity was reported recently (Dettmar *et al.*, 2011).Yield and viscosity of the alginates extracted in the present study showed lesser value for same species collected from other regions of Gulf of Mannar (Thomas & Subbaramaiah, 1991; Umamaheswara Rao, 1969). But it was more less similar to other species reported from the same region (Kalimuthu *et al.*, 1991).

Viscosity of alginate is important for its biological property. (Torres et al., 2007) investigated the in vivo anti-tumor activity of two alginates with different viscosity extracted from brown seaweed Sargassum vulgare C Agardh, against Sarcoma 180cells transplanted in mice. However, only SVLV led to acute tubular necrosis suggesting that the observed anti-tumor activity could be related to alginates immunomodulatory properties. In the present study the seaweeds analyzed showed low viscosity when compared to other seaweeds (Gupta & Abu-Ghannam, 2011; Rengasamy et al., 2003; Jothi saraswathi et al., 2006). Increase in the yield was observed at 5 hours of extraction period whereas only a slight increase in viscosity was observed in the present study. Similar effect of extraction time and temperature on specific viscosity was reported by Torres et al (2007). This was probably due to dissolution of high molar mass macromolecules that result in high solution of viscosity.

#### CONCLUSION

It can be concluded that seaweeds selected in the present study can be utilized as a source of natural antioxidant compounds as their crude extracts exhibits good antioxidant activity. Bioactive compounds found in seaweeds await a major breakthrough for a variety of food/medical applications (alginate/phenolics) as they have the potential for application as natural antioxidants in different food/ pharmaceuticals products.

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