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Evaluation on Antioxidant Activity of *Sargassum Wightii* Greville Ex J. Agardh

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ABSTRACT:

The aim of the present study was to evaluate Sargassumwightii Greville ex J. Agardh for its antioxidant activity. Two different solvent extracts namely methanol and ethyl acetate were used for the study. Three different assays such as DPPH, Hydroxyl Radical Scavenging activity and Total antioxidant activity assays were used for the study. The results of the study showed a significant activity in all the studied assays. The ethyl acetate extract showed higher antioxidant activity than methanol extract. As the concentration increases there was a remarkable increase in radical scavenging activity.

Keywords: Antioxidant Activity, DPPH, Extracts, Free radicals, Secondary metabolites.

1. Introduction

Plants during unfavorable conditions such as extreme temperature, drought, heavy metals, nutrient deficiencies and high salinity generate high concentration of free radicals (ROS), which can cause oxidative stress (Chaves *et al*., 2020).

Oxidative stress and generation of Reactive Oxygen Species (ROS) are involved in patho-physiological process causing various diseases such as cancers, arthritis, Parkinson's diseases, gastrointestinal diseases, Alzheimer's disease, aging, cardiovascular diseases, diabetes, neurodegenerative diseases. Various structural defects at the mitochondrial DNA level and functional alteration of several enzymes and cellular structures also occurs (Liguori*et al*., 2018; Sharifi-Rad *et al*., 2020).

Free radicals are unstable chemical molecules that cause damage to lipid, cells, proteins and DNA resulting in an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzyme (Kumar and Raju, 2017). Antioxidants can be considered as an agent that can protect living cells from the aggression of free radicals (Son *et al*., 2022).

Since, there is concern of synthetic antioxidants consumption due to their instability and possible activity as carcinogens. Natural antioxidants have attracted considerable attention by researchers and consumers (Xu*et al*., 2017). Seaweed is one of the most known sources for functional foods and pharmaceutical constituents with antioxidant activity (Olasehinde*et al*., 2019). It was thought to be a source of bioactive chemicals since they create a wide range of secondary metabolites such as alkaloids, phenols, flavonoids, saponins, steroids and related active metabolites with a wide range of biological activity (Sangiya and Kumar, 2022).

Sargassum inhabits the ocean basins of the Atlantic, Pacific and Indian Oceans, mostly in tropical and subtropical environments where it forms dense submarine forests (Mattio and Payri, 2011). Even though, *Sargassum* had been studied extensively for its antioxidant potential *in vitro* (Lim *et al*., 2002; Santoso*et al*., 2004; Kim *et al*., 2005; Park *et al*., 2005; Cho *et al*., 2007; Zubia*et al*., 2008; Budhiyanti*et al*., 2011), the present study was aimed to explore*Sargassumwightii*that was collected from the coastal area of Arockiapuram for its antioxidant activity.

2. Materials and Methods

Collection, Identification and Preparation of Seaweed Extracts

The collected samples Sargassumwightii were collected from the coastal region ofArockiapuram, Kanniyakumari District and identified by algal experts. The samples were initially washed with sea water to remove the macroscopic epiphytes, sand particles and other extraneous matter and then rinsed thoroughly in distilled water. The washed sampleswere air dried in shady place for 15 days and ground to fine powder using an electric mixer and were then stored in an airtight container in the refrigerator at 4ºC for further analysis.

The dried powdered samples (50gm) were immersed separately in 100 ml of different organic solvents (methanol and ethyl acetate) in a separate airtight conical flask for 2 days for the successive extraction of crude extracts. The extracts was first filtered through a double-layered muslin cloth and then filtered through Whatmann No.1 filter paper to separate the filtrate. Then, the extracts were concentrated using a vacuum rotary evaporator at low temperature (40ºC) and the filtrate was collected into sterile airtight bottle and stored in a refrigerator for further use (Arokiyaraj*et al*., 2009; Rebecca *et al*., 2012).

Antioxidant Studies

Dpph Radical Scavenging Assay

Different concentrations such as 12.5 µg/mL - 200 µg/mL from a stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1 mM) solution was added. A control without the test compound was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control (Chang *et al*., 2001).

Hydroxyl Radical Scavenging Assay

From a stock concentration of 10mg/mL were mixed with 500µl reaction mixture (2 deoxy 2 ribose (2.8mM) $FeCl_3$ (100 µm), EDTA (100 µm), H_2O_2 (1.0 mM), ascorbic acid (100µm) in KH_2PO_4-KOH buffer (20 mMpH7.4) was made up to a final volume of 1ml and different concentrations such as 125 µg/mL - 2000 µg/mL were taken. A control without the test compound was taken. After incubation for 1 hour at 37ºC, add 1ml of 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90ºC for 15 minutes to develop the colour. After cooling the absorbance was measured at 532nm against an appropriate blank solution (Elizabeth and Rao, 1990).

Total Antioxidant Assay

The stock concentration of 10mg/mL was obtained with 3ml of reagent solution (0.6ml H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) was taken. The different concentrations such as 125 µg/mL - 2000 µg/mL were taken from the stock concentration. The tube containing the reaction solutions were incubated at 95ºC for 90 minutes. The absorbance of the solution was measured at 695 nm against blank after cooling to room temperature (Methanol 0.3 ml) in the place of extract was used as blank. Ascorbic acid (10mg/mL DMSO) was used as reference. The antioxidant activity is expressed as number of gram equivalent of ascorbic acid (Prieto *et al*., 1999).

3. Results and Discussion

An antioxidant is a substance that prevents or delays the adverse effects caused by free radicals and protect our body from free radicals associate diseases (Halliwell, 1999; Alam*et al*., 2020). In the present study, antioxidant potential of *Sargassumwightii* was evaluated by DPPH, Hydroxyl radical scavenging and Total antioxidant activity assays. Two different solvent extracts like methanol and ethyl acetate were chosen for the study. Different concentrations were used to determine the antioxidant activity. All the solvent extracts showed significant antioxidant activity. Previous studiesalso reported the antioxidant activity of Sargassumwightii (Rajivgandhi et al., 2021;Vijayan et al., 2018; Deepak et al., 2017; Kumar and Raju, 2017; Sujatha et al., 2017; Yuvaraj and Arul, 2014; Indu and Seenivasan, 2013)

The DPPH radical scavenging capacity were evaluated by scavenging of 2,2diphenyl-picryl-hydrazyl (DPPH) free radical. The DPPH assay was used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors (Singh and Rajini, 2004). DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger (Budhiyanti*et al*., 2012). The ethyl acetate extract exhibited a strong DPPH activity of 53.28±0.43% inhibition at 200 µg/mL with the IC_{50} value of 160.86 µg/mL. The methanol extract showed 52.31±0.88% of inhibition with the IC_{50} value of 165.46 µg/mL.The IC_{50} value is defined as the concentration of the sample to inhibit oxidation up to 50% or the concentration of the test sample to capture 50% of free radicals. According to Palanisamy*et al*. (2018), smaller IC_{50} value indicates stronger antioxidant ability.Prior and Cao (1999) suggest that the lower the IC_{50} value, the higher the

antioxidant activity of antioxidants. This was due to the use of lower concentrations can already inhibit free radicals by 50%.

Several authorsreported that the brown algae,*Sargassum* species showed good DPPH free radical scavenging activity against DPPH molecule (Vinayak*et al*., 2011; Zahra *et al*., 2007; Wei *et al*., 2003; Toth and Pavia, 2000). This result of the previous studies correlates with our present investigation.

Hydroxyl radical scavenging activity is based on the qualification of the degradation product of 2-deoxyribose by condensation with Thiobarbituric acid (TBA) (Nair *et al*., 2016). It is the most reactive oxygen centered species and cause severe damage to adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron Ethylenediaminetetraacetic acid (EDTA) (Pavithra and Vadivukkarasi, 2015). The maximum scavenging activity was exhibited in the ethyl acetate extract with the percentage of inhibition of 55.26±0.97% at 2000 µg/mL concentration. The IC_{50} value of ethyl acetate extract was 1495.18µg/mL. The methanol extracts showed the scavenging activity with percentage of inhibition of 54.54 ± 1.03 % with the IC_{50} value of 1524.86 µg/mL. The scavenging activity was slightly higher in Hydroxyl scavenging assay than DPPH assay.

The seaweed sample extracts were also assessed for Total antioxidant activity. From the two different solvent extracts, the ethyl acetate extract exhibited high Total antioxidant activity with the percentage of inhibition of 54.88±0.71% with the IC_{50} value of the extract was 1615.42 µg/mL. The methanol extract showed the inhibition percentage of 54.79±0.77% with the IC_{50} value of 1642.68 µg/mL at 2000 µg/mL concentration.

The results of the assays in the present study showed a large difference between radical scavenging activities of two different solvent extracts. The ethyl acetate extract showed better antioxidant potential than methanol extract. Maryam *et al*. (2013) reported that the ethyl acetate extracts exhibited better antioxidant activity than methanol extracts. This study indicated that the ethyl acetate extract of seaweeds had better antioxidant effect in comparison with methanol extracts. Among all the tested antioxidant assays, Hydroxyl radical scavenging showed higher scavenging activity.Indu and Seenivasan (2013) and Kumar*et al*. (2008) reported that the hydroxyl radical scavenging activity by the brown algae, *Sargassumwightii* and *Sargassummarginatum*was found to be higher which correlates with our present investigation.

The antioxidant efficacy of extracts increased with increasing concentration in all the assays studied, showing that these properties are dose dependent. Previous reports showed that the antioxidant activities of extracts of all the four seaweeds (*Padinapavonica, Colpomeniasinuosa, Cystoseiramyrica*and *Ulvalactuca*) exhibited dose dependency and the activity increased with increasing concentration of the extract (Maryam *et al*., 2013).

The results of the experiment indicated that the ethyl acetate extract of *Sargassumwightii* possessed significant amount of antioxidant activity. Further studies are required to elucidate the activity principle of this extract; thereby it can be used as therapeutic agent.

Table.1. DPPH Radical Scavenging Activity of Two Different Solvent Extracts of Sargassumwightii

Concentration µg/mL	**Percentage of Inhibition**		
	Methanol	Ethyl Acetate	Standard (Ascorbic acid)
12.5	16.13±0.17	14.39±0.12	26.65±0.53
25	26.48±0.83	23.77±0.97	43.67±0.67
50	34.76±1.54	32.08±0.52	56.87±0.55

100	44.41±1.77	45.91±1.63	78.83±0.74
200	52.31±0.88	53.28±0.43	93.91±0.63

Figure.1. DPPH Radical Scavenging Activity in Methanol and Ethyl Acetate Extract of Sargassumwightii

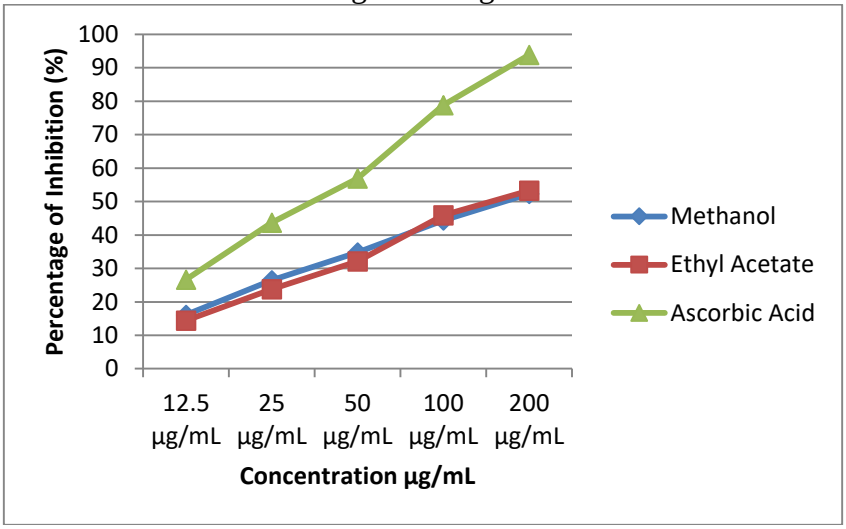


Table.2. Hydroxyl Radical Scavenging and Total Antioxidant Activity of Two Different Solvent Extracts of Sargassumwightii

Concentration µg/mL	**Percentage of Inhibition**					
	Hydroxyl Radical Scavenging Activity			Total Antioxidant Activity		
	Methanol	Ethyl Acetate	Standard (Gallic acid)	Methanol	Ethyl Acetate	Standard (Ascorbic acid)
125	9.57±0.34	12.83±0.77	20.03±0.01	8.27±1.23	13.11±0.21	23.74±0.09
250	24.06±0.65	24.78±0.97	50.12±0.19	16.21±0.45	20.67±0.97	48.58±0.22
500	38.02±0.21	37.62±0.63	64.86±0.30	30.73±0.28	36.04±0.66	63.14±0.05
1000	46.47±0.55	47.28±1.01	77.93±0.15	39.75±0.66	43.98±0.35	74.48±0.06
2000	54.54±1.03	55.26±0.97	93.89±0.08	54.79±0.77	54.88±0.71	93.29±0.56

Figure.2. Hydroxyl Radical Scavenging Activity in Methanol and Ethyl Acetate Extract of Sargassumwightii

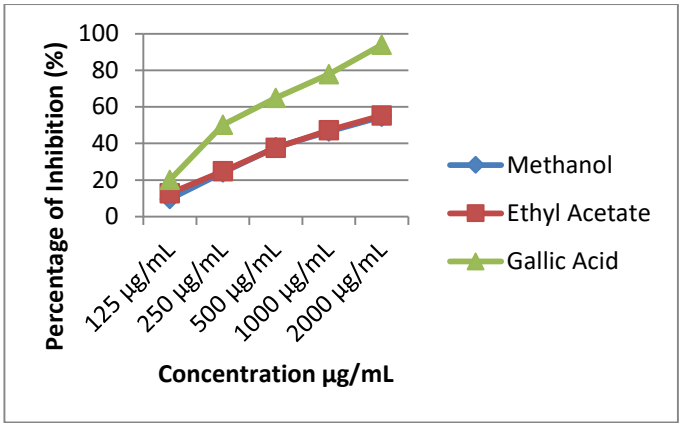


Figure.3. Total Antioxidant Activity in Methanol and Ethyl Acetate Extract of Sargassumwightii

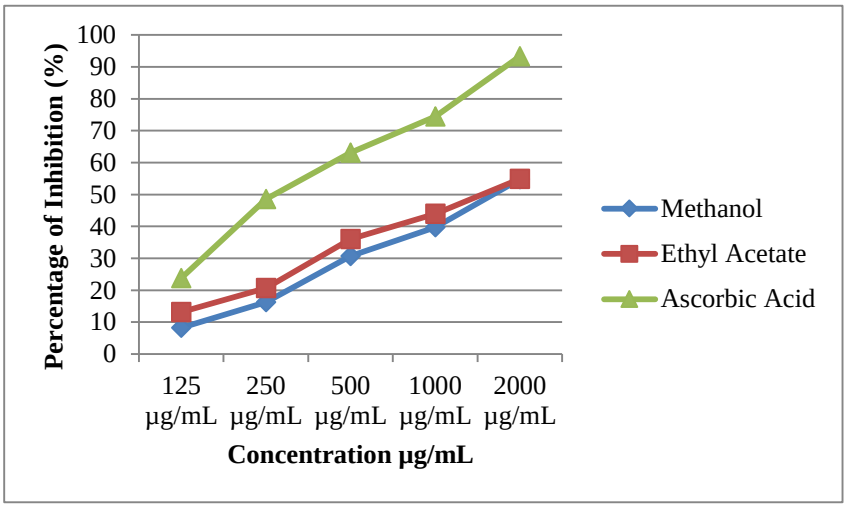
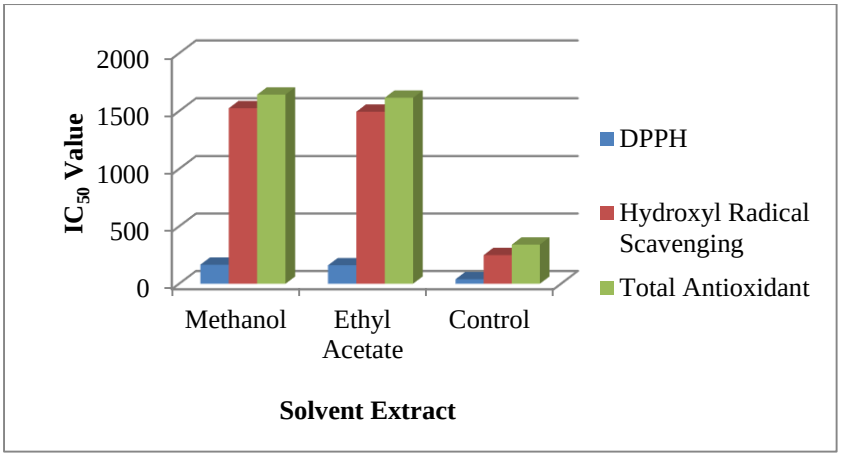


Table.3. IC_{50} Value of Different Solvent Extracts of *Sargassumwightii* from the Antioxidant Activity Assay

Antioxidant Activity	**IC_{50} value**		
	Methanol	**Ethyl acetate**	**Control**
DPPH	165.46 µg/mL	160.86 µg/mL	39.09 µg/mL
Hydroxyl radical scavenging	1524.86 µg/mL	1495.18 µg/mL	249.46 µg/mL
Total antioxidant	1642.68 µg/mL	1615.42 µg/mL	340.93 µg/mL

Figure.4. IC_{50} (µg/mL) value of Different Solvent Extracts of *Sargassumwightii*



4. Conclusion

From the present study, the results of the *in vitro* antioxidant activities of two different solvent extracts of *Sargassumwightii* showed significant antioxidant potential. The ethyl acetate extract showed a strong antioxidant activity than methanol extract. Therefore, ethyl acetate extractof *Sargassumwightii*may be considered as a potential source of natural antioxidants for therapeutic or industrial purpose and as alternative for the synthetic products.

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