ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.1.L60-67

International Journal of Lifescience and Pharma Research

Research Article

Pharmaceutical for effective drug dosage

Evaluation of Antioxidant Property in Secondary Metabolites Isolated from *Sargassum wightii* Collected From Kanyakumari Coastal Region, Tamilnadu.

Vellingiri Manon Mani[1&2*], Selvam Nivethitha[2], Kathirvel Preethi[2], A. Parimala Gnana Soundari[2&3], V. Subha Priya[1], P. Deepak[1] and M. P. Ayyappa Das[1].

[1] *Department of Biotechnology, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamil Nadu, India.*

[2]*Department of Microbial Biotechnology, Bharathiar University, Coimbatore- 641046, Tamil Nadu, India.*

[3]*Department of Environmental Science & Engineering, College of Natural Resources & Environment, Northwest A & F University, Shaanxi- 712100, China.*

Abstract: The demands for increasing anticancer drugs produced by natural sources with potential antioxidant and antimicrobial properties had led the path to mass production in pharmaceutical fields. This current investigation is implied to extract the potential crude secondary metabolites extract from brown algae *Sargassum wightii* Greville ex J.Agardh with maximum antimicrobial and antioxidant properties. The brown seaweed was collected from Kanyakumari, Tamil Nadu, India and taken for extraction process using various polar and non-polar solvents. The solvents which extracted maximum crude metabolites were taken to determine the preliminary antimicrobial analysis using clinical pathogens on which the crude extracts explored the highest zone of inhibition at least concentration. Further, the five different crude extracts were subjected to antioxidant assessment and the extract in different solvents manifested maximum activity at minimum concentration. The results of antimicrobial and antioxidant analysis evinced that the ethyl acetate and methanolic extract exhibited maximum activity when compared to other crude extracts. The extract which explored the highest activity was taken for qualitative and quantitative phytochemical determination and both the extract showed the presence of most of the phytochemicals. Further, the investigation is carried out to purify and determine the anticancer property in cancer cell lines.

Keywords: Anticancer, antioxidant, antimicrobial, DPPH, marine algae, seaweeds.

***Corresponding Author**

Vellingiri Manon Mani,Kathirvel Preethi, , Department of Biotechnology, Rathnavel Subramaniam College of Arts and Science, Coimbatore- 641402, Tamil Nadu, India, Department of Microbial Biotechnology, Bharathiar University, Coimbatore- 641046, Tamil Nadu, India.

Recieved On 11 November 2019
Revised On 06 December 2019
Accepted On 10 January 2020
Published On 02 April 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Vellingiri Manon Mani, Selvam Nivethitha, Kathirvel Preethi, A. Parimala Gnana Soundari, V. Subha Priya, P. Deepak, M. P. Ayyappa Das. , Evaluation of antioxidant property in secondary metabolites isolated from Sargassum wightii collected from Kanyakumari coastal region, Tamilnadu..(2020).Int. J. Life Sci. Pharma Res.10(1), L 60-67 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.1.L60-67

1. INTRODUCTION

Algae are a large and diverse group of simple plant like organisms ranging from unicellular to multicellular and macrothallic forms. The largest and most complex marine algae are called seaweeds. Seaweeds are a group of macroscopic marine algae that form the biomass in the intertidal zone and the term seaweeds and sea vegetables are used interchangeably [1]. Seaweeds are salt water tolerant, land dependent plants growing almost exclusively at narrow interface where land and sea meet. The seaweeds are always photosynthetic and for their growth needs abundant sunlight. Seaweeds are found abundantly along the Tamil Nadu and Gujarat coastal lines and around Lakshadweep and Andaman and Nicobar islands. These are also called as benthic sea algae which predominant attached along the sea. Seaweeds are macrophytic algae, a primitive type of plants lacking true roots, stems and leaves. Most seaweed belongs to one of the three divisions namely Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Mostly brown algae were found to have many futuristic properties and have been used mostly in medicinal fields to treat various diseases. *Sargassumwightii*, belonging to the family Sargassaceae is an abundant marine brown alga commonly found in the shorelines of India. It is a macroscopic, multicellular, photosynthetic, non-vascular, pelagic marine species rich in sulphated polysaccharides that manifest potent free radical scavenging [2] and antioxidant effects[3].Carbohydrate, steroids, flavonoids, phenols, terpenoids and sulphated polysaccharide, a potential natural antioxidant which are not found in land plants property [4] in addition to vitamins and minerals, highest amount of protein and lipid in seaweeds are most adequate for consumption compared to other land vegetables mainly due to their high content in essential amino acid and relatively high level of unsaturated fatty acid. [5] Arunkumar *et al.*[6] has reported the presence of sulphoglycerolipid 1-0-palmitoyl-3-0 (6-sulfo –α-quinovicpyranosyl) -glycerol isolated from the methanolic extract of the brown seaweed *Sargassumwightii* inhibited the growth of *Xanthomonasoryzae*pv.*oryzae* which cause bacterial blight of rice. There are no investigation on the different solvent extracts of *S. wightii* exposed for its activity against treatment for cancer and the present study was carried out to evaluate the presence of phytochemical and biochemical constituent and also to determine the antioxidant and antimicrobial properties for crude extract of *S. wightii.*

2. MATERIALS AND METHODS

2.1. Collection of macroalga

A macro alga (seaweed) was collected from the coastal area of Kanyakumari, Tamil Nadu, India (Latitude- 0.0780^{0}N and Longitude- 77.5410^{0}S) during the month of May 2015. The seaweed was washed with sea water and fresh water thoroughly to remove the epiphytes and other contaminants. The algae was transported to the laboratory and air dried in shade (to avoid thermal degradation of the metabolites) for 7 days. The air dried macroalgae was coarsely powdered and stored in refrigerator at 4°C for further studies.

2.2. Identification of algal sample

Algal species was identified according to Chapman and Gellenbeck[7]. Taxonomic classification of the algal species was made according to the system developed and modified by Papenfuss[8]. The identification was based on (a) morphological, using external and internal characteristics and (b) ecological, by distribution and habitat.

2.3. Preparation of extracts

The pulverized moisture free seaweed materials (25 mg) were immersed in 150 ml of various solvents like methanol, ethanol, ethyl acetate, acetone, acetonitrile, DMSO, chloroform, petroleum ether and hexane separately. The extraction was carried out in a shaker for 24 hrs and this process was repeated twice for the extraction of metabolites from the seaweed. The weight of the extracts was calculated and the yield percentage (%) was determined after concentrating the extracts.

2.4. Antimicrobial activity for crude extracts

The antimicrobial activity was performed by well diffusion method [9] against the test organisms such as *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella*sp, *Salmonella* sp, *Proteus* sp, *Shigella*sp, *Bacillus* sp, and *Candida albicans*. By using a sterile cork borer, wells were punctured in Mueller Hinton agar plates previously swabbed with one of the test organisms. About 40µl of algal sample extract was added in each well. The diameters of inhibition were determined after 24h of incubation at 37^{0}C for bacteria, and after 48h at 28 ^{0}C for fungi[6].

2.5. Preliminary antioxidant screening for crude extracts

2.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity was determined by using different concentration (20µg/ml to 100 µg/ml)of sample from each extract (methanol, ethyl acetate, DMSO, chloroform, hexane), and were made up to 1 ml with methanol and to this 0.1mM methanolic DPPH solution was added and vortexed well. The mixture was incubated at 27°C for 20 mins. 0.1mM methanolic DPPH solution served as control and absorbance of samples were measured at 517 nm using methanol (Blank) to set 0 [10]. The ability of the sample to scavenge DPPH radical was calculated by the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

2.5.2 *Reducing power*

Total reducing power was determined as described by Mani *et al.*[10].1 ml of sample, at different concentrations (20µg/ml to 100 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 mol/ L, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) was taken. The mixture was incubated at 50°C for 20 mins, 2.5 ml of trichloro acetic acid (10% TCA) was added to the mixture and centrifuged at 3000g for 10min. The culture filtrate of about 5 ml was mixed with a ml of 0.1% of ferric chloride, and the optical density was measured at 700 nm in a Spectrophotometer.. The crude extracts which exhibited maximum antioxidant activities were taken for further studies.

2.6. Qualitative phytochemical screening

The following tests were performed on different extracts to detect various phytoconstituents using standard protocols [11].

2.7 Quantitative phytochemicals screening

2.7.1 *Total phenolic content*

Total phenolic assay was determined by using Folin-Ciocalteu assay[11]. A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot (1ml) of extract or standard solution of gallic acid was added to 250ml of flask containing 9ml of distilled water. 1ml of Folin- Ciocalteu phenol reagent was added to mixture and shaken. After 5 minutes 10ml of 7%sodium bi carbonate was added. The solution was diluted to 25ml with distilled water and mixed. After incubation for 90 minutes at room temperature, the absorbence was determined at 750nm with UV spectrophotometer. Total phenolic content was expressed in terms of gallic acid equivalent (mg/g of extracted compound).

2.7.2. *Estimation of flavonoids*

Total flavonoid content was measured by the Aluminum chloride colorimetric assay [11]. A known amount of the sample was taken; grounded well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot 1ml of extract or standard solution of catechin (20, 40, 60, 80 &100µg/ml) was added to 10ml volumetric flask containing 4ml of double distilled water. To the flask was added 0.3ml 5% sodium nitrate, after 5 mins, 0.3ml of 10% aluminum chloride was added. At sixth minute, 2ml of1M NaOH was added and the total volume was made up to 10mlwith double distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg rutin equivalents (CE)/100g dry mass samples were analyzed in duplicate.

3. RESULTS

3.1 Identification of algae

The collected alga was identified as *Sargassum wightii*. The alga was dark-brown, 20-30 cm in height with a well-marked holdfast, upper portion richly branched, axes cylindrical, glabrous, leaves 5-8 cm long and 2-9 mm broad, leaves tapering at the base and apex, midrib inconspicuous vesicles large, spherical or ellipsoidal being 5-8mm long and 3-4 mm broad, stripe of the vesicle 5-7 mm long seldom ending into a long tip, receptacles in clusters and repeatedly branched.

Fig. 1.Morphology of *S. wightii*

Brown alga collected from Kanyakumari, Tamilnadu, India.

3.2. Extraction of crude metabolites

The crude secondary metabolites were extracted using ten different nonpolar and polar solvents. The extractions of metabolites were observed through UV-VIS spectrophotometric analysis and the highest absorbance of 2.47, 2.89, 2.75, 2.94, 2.73 was recorded in hexane, chloroform, DMSO, methanol and ethyl acetate, as given in Fig. 1. Among the ten solvent extractsonly five extracts viz hexane, chloroform, DMSO, methanol and ethyl acetate have given the highest peak value which was given in Fig. 2. Thus these five solvent extracts were taken for further studies.

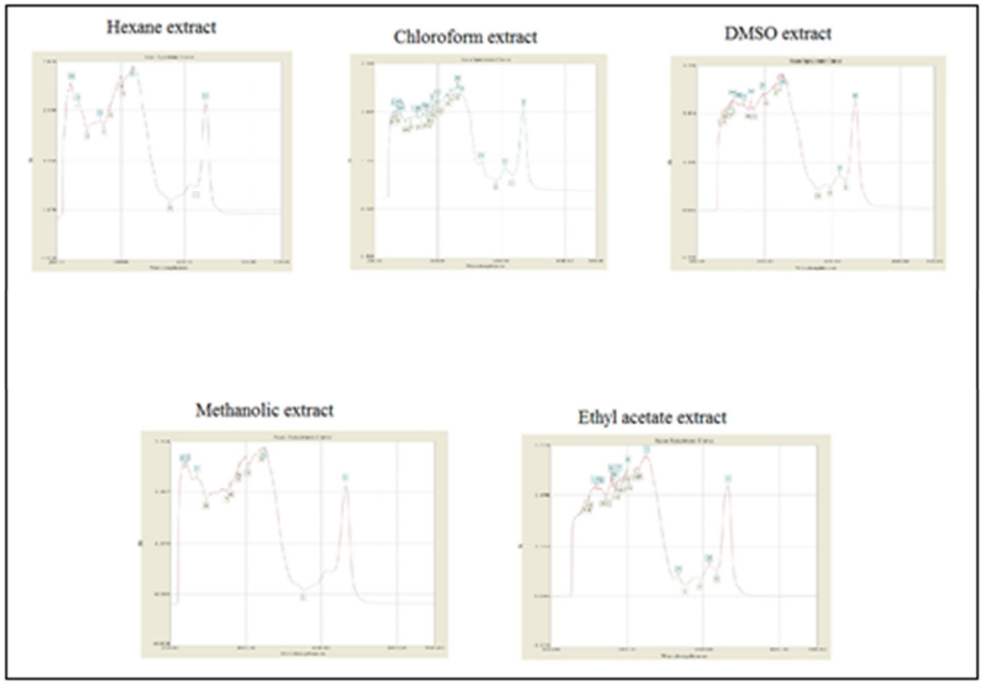

Fig. 2. UV-VIS spectrophotometric analysis of crude extract in different solvents

Hexane, chloroform and DMSO: non polar solvents; ethyl acetate and methanol: polar solvents.

3.3 Antimicrobial activity for crude extracts

The antimicrobial activity of the crude extracts was performed and the results were observed. The ethyl acetate, methanol and chloroform extract was inhibiting the growth of *Pseudomonas* sp at 1.5cm, 2cm and 1cm respectively (Table.1). The *Shigella* sp was highly susceptible to ethyl acetate, methanol and chloroform extract and showed inhibition of 1.2cm, 1.3cm and 1.5cm respectively. On comparing with the five different crude extracts, hexane, and DMSO extracts did not inhibit the growth of tested pathogens but methanolic and ethyl acetate extracts were found to be inhibiting most of the tested pathogens.

Table. 1: Antimicrobial activity of different crude extracts from *S. wightii*

S.No	Name of the extracts	Pathogens	Zone of inhibition (diameter in cm)
1	Methanol	*S. aureus*	1
		S. epidermidis	1.2
		Klebsiella sp	1
		Shigella sp	1.3
		S. typhi	1
		P. aeruginosa	1.5
		E. coli	1.2
		V. cholera	1
		Proteus sp	1.4
		C. albicans	1
2	Ethyl acetate	*S. aureus*	1.5
		S. epidermidis	1.4
		Klebsiella sp	1
		Shigella sp	1.2
		S. typhi	1.4
		P. aeruginosa	2
		E. coli	1.5
		V. cholera	1.7
		Proteus sp	1.2
		C. albicans	1.5
3	Chloroform	*S. aureus*	1
		S. epidermidis	1.2
		Klebsiella sp	1
		Shigella sp	1.5
		S. typhi	1

		P. aeruginosa	1
		E. coli	1.2
		V. cholera	1
		Proteus sp	1.3
		C. albicans	1
4	DMSO	*S. aureus*	0.7
		S. epidermidis	-
		Klebsiella sp	0.9
		Shigella sp	0.8
		S. typhi	1.1
		P. aeruginosa	1.2
		E. coli	-
		V. cholera	-
		Proteus sp	-
		C. albicans	0.8
5	Hexane	*S. aureus*	-
		S. epidermidis	-
		Klebsiella sp	-
		Shigella sp	1.1
		S. typhi	0.7
		P. aeruginosa	-
		E. coli	-
		V. cholera	0.8
		Proteus sp	0.9
		C. albicans	-

<0.5 cm indicates no zone of inhibition

3.4 Antioxidant activities of the crude extracts

3.4.1 DPPH radical scavenging assay

Based on the activity of the crude extracts (different crude extracts) IC_{50} value was observed highest in ethyl acetate and methanolic extract. The IC_{50} concentration of DMSO, ethyl acetate, methanol, hexane and chloroform extracts were found to 24, 49, 52, 22 and 30µg/ml respectively. The activities of different extracts at various concentrations were given in graph (Fig. 3).

3.4.2 Reducing power assay

The reducing capacity of the extracts (DMSO E, EAE, ME, HE and CE) may serve as a significant indicator of potential antioxidant activity. The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) was measured by the intensity of the resultant Persian-blue solution at 700 nm. The reducing power of five different crude extracts exhibited increased OD units with increasing concentration and among the five extracts, ME and EAE was found to exhibit the highest OD units at a lower concentration. The results were displayed in Table (2). The result showed that extracts may consist of hydrophilic compounds that cause greater reducing power. The results of the antimicrobial and antioxidant assessment of five different crude extracts exhibited the highest activity in methanolic (ME) and ethyl acetate crude extract (EAE). Further, these two crude extract (ME and EAE) were taken for studies.

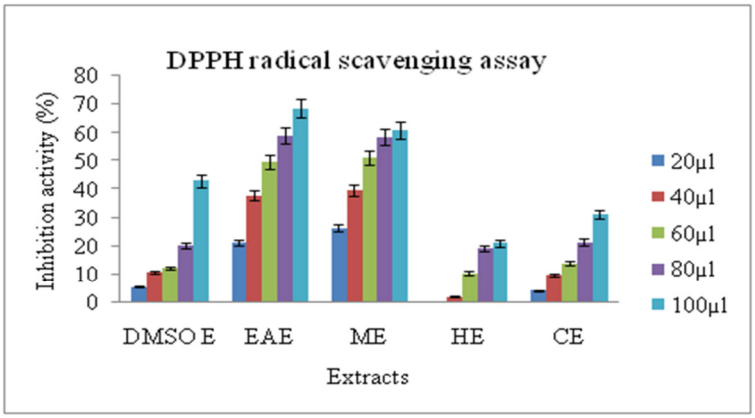

Fig. 3. DPPH radical scavenging activity for five different crude extracts

Data represented as mean value± SD (n=3). DMSO E: DMSO extract; EAE: Ethyl acetate extract; ME: Methanol extract; HE: Hexane extract; CE: Chloroform extract; Standard: Ascorbic acid.

Table.2: Reducing power assay for different crude extract						
	Optical Density units					
Concentration (µg/ml)	DMSO E	EAE	ME	HE	CE	Standard
20	0.101±0.03*	0.162±0.04*	0.178±0.03*	0.071±0.05	0.075±0.5	0.171±0.03
40	0.119±0.04	0.210±0.03	0.256±0.5	0.087±0.03	0.083±0.05	0.258±0.02
60	0.135±0.4	0.431±0.03	0.526±0.03*	0.148±0.03	0.124±0.06	0.498±0.06*
80	0.189±0.03	0.543±0.06*	0.621±0.5*	0.169±0.06	0.147±0.03*	0.618±0.03
100	0.183±0.02*	0.242±0.04*	0.421±0.06	0.225±0.04*	0.526±0.03	0.321±0.02*

Data represented as mean value ± SD (n=3). DE: DMSO extract; HE: Hexane extract; CE: Chloroform extract; EAE: Ethyl acetate extract; ME: Methanol extract; Standard: Ascorbic acid. *p<0.05: significant.

3.5. Qualitative phytochemical screening

The preliminary phytochemical studies on hexane, DMSO, chloroform, methanol and ethyl acetate extracts of *S. wightii* revealed the positive results for some phytochemicals (Table.3). The positive results exhibited the presence of steroids, phenolic compounds, saponins, tannins and flavonoids. Among the five solvent extracts used for the phytochemical analysis, only methanol and ethyl acetate showed the presence of maximum compounds. Hence, these two solvents were taken for further studies.

Table. 3: Phytochemical analysis for crude extract by using different solvents						
S.No	Phytochemical constituents	Name of the solvents				
		Sargassum wightii				
		Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	DMSO Extract
1	Alkaloids	+	-	+	+	-
2	Flavonoids	+	+	+	+	-
3	Phenols	-	-	+	+	+
4	Tannins	-	+	+	-	+
5	Cardiac glycosides	-	+	+	+	+
6	Steroids	-	-	+	-	+
7	Saponins	-	+	+	-	+
8	Terpenoids	-	-	-	-	+

'+' denotes presence of phytochemicals; '-' denotes absence of phytochemicals.

3.6. Quantitative phytochemical screening

3.6.1 Estimation of total phenolics

The determination of the total phenol content was carried out using gallic acid as standard. Phenols are well known compounds, owing to potent antioxidant activities and bioactivities, are well known to diffuse free radicals [12]. In the present work, *S. wigtii* was examined for its total phenolic content using Folin-Ciocalteu reagent method. The content of total phenolic in *S. wigtii* was given in the Table. 4. The phenolic content was higher in methanolic solvent extraction when compared with ethyl acetate extraction.

Table. 4: Quantitative phytochemical analysis.			
		Estimated values	
S.No	Parameters	ME	EAE
1	Phenol (µg galic acid /mg of the extract)	2.176±0.070	1.185±0.0070
2	Flavonoids (µg rutin /mg of the extract)	3.326±0.200	2.176±0.070

ME: Methanol crude extract; EAE: Ethyl acetate crude extract

3.6.2. Estimation of total flavonoids

Rutin was used as standard for the determination of total flavonoid content. Flavonoids are considered to be strong scavengers of ROS [13].The total flavonoid content was measured by aluminium colorimetric method and was found to be 3.326±0.200 (ME) and 2.176±0.070 (EAE) µg rutin /mg of the extract.

4. DISCUSSION

The modern research has been focused on the extraction of natural products for the field of medicine and pharmaceutical to treat various diseases and disorders. Majorly the natural products are derived from plants and microorganisms as secondary metabolites- a bioproduct. This emergence has led the researchers to extract the bioproducts from different sources such as seaweed (marine algae). This current investigation has been carried out to explore the biological nature of a secondary metabolites extracted from a potential seaweed/ marine algae, *S. wightii.* This particular seaweed has been reported with many biological properties but this was collected from a different source with different climatic conditions. So the properties may differ accordingly with the habitat of the source. This research was done to evaluate the antimicrobial and antioxidant properties for the crude metabolites extract and to also determine the phytochemical

constituents. The crude metabolites were extracted using different solvents to find out the best solvent which extracts maximum metabolites. The solvents which extracted maximum crude metabolites were assessed for antimicrobial and antioxidant assessment and this would result in the complete crude extract with highest biological properties. The antimicrobial assessment exhibited a strong activity against most of the tested pathogens in this study. This result was in accordance with the investigation of Savita[14] which revealed that the methanolic extracts of 17 commonly found seaweeds in the west coast of India were screened for the presence of antimicrobial activity against *S. aureus, S. epidermidis*, *Klebsiella*sp, *Shigella* sp, *S. typhi, P. aeruginosa, E. coli, V. cholerae, Proteus* sp, *C. albicans*. Further the study of Veeragurunathan et al [15] stated that brown algae explored the maximum antibacterial activity against eight pathogenic strains. The determination of antioxidants was done using two main scavenging assays; DPPH and reductive power. Ability to scavenge DPPH free radicals shows its effectiveness in prevention and repair mechanism against injury in a biological system [16]. Scavenging of DPPH represents the free radical reducing activity of antioxidants based on one electron reduction. The reduction in the number of DPPH molecules can be correlated with the number of available hydrogen groups. The five different solvents taken for this analysis and among that five ethyl acetate extract and methanol extract evinced a promising antioxidant activity at least concentration. Further the reducing power was analyzed and similarly ethyl acetate and methanolic extract explored the maximum activity at minimum concentration. So these two extracts were taken for further studies to determine the presence of phytochemicals. The methanolic and ethyl acetate extract showed the presence for maximum phytochemicals when compared to other extracts. The quantification analysis exhibited highest range of phenols, flavonoids and proteins in a similar range and this investigation was similar to the research of Veeragurunathan*et al*[15]. The poly phenols include large subgroups of chemicals called flavonoids and they are plant byproducts found in a broad range of fruits, grains and vegetables. They are being studied to find out whether they can prevent chronic diseases such as cancer and heart diseases. These are thought to eliminate the harmful molecules known as free radicals from the body, which can damage a cell's DNA and may trigger some forms of cancer and other diseases. Flavonoids act as antioxidants and protect against oxidative stress [17]. Janarthanan and Senthilkumar[18] reported that the phenol content was highly presented in *S. wigtii* and the result of the stated report coincides with the current study. Further, the crude metabolites extract will be taken for purification and structural elucidation analysis and the anticancer studies will be evaluated.

5 CONCLUSION

The current investigation focused to develop the antioxidants for medicinal applications from seaweeds. The seaweeds mainly *S. wightii* brown algae are found to contain more of biological properties in minimum quantity and in that target the research aimed to produce the crude extract of metabolites containing antimicrobial and antioxidant properties at least concentration. Further, the research will focus to purify the metabolite and apply it as an anti- cancer or antimicrobial products.

6. AUTHORS CONTRIBUTION STATEMENT

Dr. V. Mano Mani conceptualized and gathered the data with regard to this work. Mrs. S. Nivethitha and Dr.K. Preethi analyzed these data and necessary inputs were given towards the designing of the manuscript. Dr. A. Parimala Gnana Soundari provided the instrumental facility towards this work. All other authors discussed the results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8 REFERENCES

1. Wong KH, Chung PCK. Nutritional evaluation of some subtropical red and green seaweeds. Part I-proximate composition, amino acid profiles and some physico-chemical properties. Chemistry. 2002; 71(4): 475-482. DOI:10.1016/S0308-8146(00)00175-8
2. Park PJ, Heo SJ, Park EJ, Kim SK, Byun HG, Jeon BT, Jeon YJ. Reactive oxygen scavenging effect of enzymatic extracts from *Sargassum thunbergii*. J Agric Food Chem. 2005; 53(17): 672. DOI: 10.1021/jf050582+
3. Xue CH, Fang Y, Lin H, Chen L, Li ZJ, Deng D, Lu CX. Chemical characters and antioxidative properties of sulfated polysaccharides from *Laminaria japonica*. J Appl Phycol. 2001;13(1): 6770. DOI: 10.1023/A:1008103611522
4. Chakraborty GS. Antimicrobial activity of the leaf extracts of *Calendula officinalis* (Linn). J Herb Med Toxicol. 2008; 2(2): 65-66.
5. Peer JK, Rath SK, Jena K, Rathod VK, Thatoi H. Evaluation of antioxidant and antimicrobial activity of seaweed (Sargassum sp.) extract: A study on inhibition of Glutathione-S Transferase activity. Turk J Biol. 2005; 32(2): 119-125. Available from: http://drs.nio.org/drs/handle/2264/1075
6. Arunkumar K, Selvapalam N, Rengasamy R. The antibacterial compound sulphoglycerolipid 1-0 palmitoyl-3-0 (6`sulpho α quinovo pyranosyl) glycerol from *Sargassum wightii* Greville (Phaeophyceae). Botanica. Marina. 2005; 48(5-6): 441- 445. DOI: 10.1515/bot.2005.058
7. Chapman DJ, Gellenbeck KW. An historical perspective of algal biotechnology. Algal and cyanobacterial biotechnology. Longman group, U.K. 1989; 1-27.
8. Papenfuss GF. Classification of the algae. In: A century of progress in the natural sciences. California Academy Science San Francisco. 1955; 115-224.
9. Cappuccino JG, Sherman P. Microbiology-a laboratory Manual. Pearson Education Inc., New Delhi, India 2004:282-3.
10. Mani VM, Soundari APG, karthiyaini D Preethi K. Bioprospecting for endophytic fungi and their metabolites from medicinal tree *Aeglemarmelos* in

Western Ghats, India. Microbiol.,43(3): 303- 310. DOI: 10.5941/MYCO.2015.43.3.303.
11. Preethi K, ShunmugaPiramu T. Phytochemical analysis and antioxidant activity of *Coralline officalines* and *Turbinaria ornate* seaweeds from thoothukudi coastal area. Int J Bioscience Res. 2013.2 (3): 12-17.
12. Willcox JK, Ash SL Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004; 44(4): 275-295. DOI: 10.1080/10408690490468489
13. Yang JE, Martinson T, Liu RH. Phytochemical profiles and antioxidant activities of wine grapes. Food. Chem. 2009; 116: 332–339. DOI: 10.1016/j.foodchem.2009.02.021
14. Savitha J. A prototype of proposed treatment plant for sago factory effluent. J Cleaner Prod. 2009; 17: 1363-1372. DOI: 10.1016/j.jclepro.2009.03.021
15. Veeragurunathan V, Vellaiyan R, Subramanian SS, Sadannantham G, Bhagyaraj R. Studies on the antibacterial activity of selected seaweeds from Gulf of Mannar. Seaweed Res. Utilin. 2008; 30 (1 &2): 147-152.
16. Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines. Thomson PDR. 3rd ed. Montvale, NJ: 2004.
17. Lee HS, Ohnishi Y, Horinouchi S. ArB-like factor responsible for carotenoid biosynthesis in *Streptomyces griseus*. J Mol Microbiol Biotechnol. 2001;3(1): 95-101.
18. Janarthanan M, Senthilkumar M. Qualitative and Quantitative Analysis of Phytochemical Studies on Selected Seaweeds *Acanthopora spicifera* and *Sargassum wightii*. Int J Eng Res Develop.2013; **7**: 11-15.