PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE CRUDE EXTRACT OF *SARGASSUM WIGHTII*

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***Abstract*: Seaweeds are marine macroscopic algae which form an important component of marine living organisms. In recent years, the secondary metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. *Sargassum wightii growing* in tropical and sub tropical regions have been known for a wide range of biological activities. The aim of the present study was to perform the phytochemical analysis of secondary metabolites both qualitatively and quantitatively of the three different extracts of *Sargassum wightii*. The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, steroids, tannins, flavonoids, proteins and phenolic compounds and saponins was found to be absent. Among the three different extracts, methanol extract showed the presence of maximum number (7) of compounds. The estimation of total phenolics, tannins and flavonoids were observed in different extracts of *Sargassum Wightii*. The results indicated that the maximum phenolic content (2.74±0.17 mg GAE/g dry wt) and tannin content (2.65±0.43 mg CAE/g dry wt) was recorded in the methanol extract of brown alga *S. wightii*. The maximum flavonoids content (1.97±0.03 mg RUE/g dry wt) was recorded in the red chloroform extract.**

***Keywords*: Seaweeds, *Sargassum Wightti*, Phytochemical Screening, secondary metabolites, Solvents**

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Introduction:

The early scientific workers in the phytochemical studies has considerable widen the scope of plant metabolites and to investigate the bioactive with marked pharmacological action and recent year interest of investigation in the plant kingdom as a potential source of new drugs and strategies for the fraction of plant extracts basing on biological active compounds rather than on a class or group of compounds. Phytochemical investigation of marine resources involves authentication, extraction and characterisation of isolated compounds in both qualitative, quantitative and assessment of their pharmacological activities[1]. Seaweeds are marine macro algae growing in tropical, subtropical shallow water of sea, estuaries comprising more than 200 species[2]. Depending upon the pigmentation and chemical compositions they are classified as Rhodophyta (red algae), Pheophyta (brown algae), Chlorophyta (green algae). Seaweeds are non-toxic and provide hundreds of organic compounds. Sargassum is a kind of brown algae belongs to family sargassaceae rich in carotinoids, fatty acids, vitamins, polysaccharides, the non-nutritive phtochemicals like carragenan and agar from algae are extensively used in pharmaceutical, textile, dairy, paper industries as gelling, stabilizing and thickening agent.[2] Very recently, scientists worldwide have paid much attention to both qualitative and quantative evaluation of marine organisms as alternative sources as novel metabolites with interesting biological and pharmacological activities[3]. The coastal region of South East India supports a rich of marine macro algae. Among the macro algae brown and were much abundance in the sea shores. Seaweeds have the capacity to synthesize diverse secondary metabolites with wide range of biological activities. It has been reported that the brown seaweeds contain important phytoconstituents which have the immense biological activities[4,5]. Phytochemicals of seaweeds having multiple mechanisms of action as antioxidant, stimulation of the immune system, rate of metabolism, as well as antimicrobial effects. Sargassum wightii is one of the brown algae contains enormous secondary metabolites with immense biological, pharmaceutical and pharmacological properties[6,7]. Hence preliminary phytochemical analysis on seaweeds will be glorious approach to estimate the bioactive secondary constituents.

Materials and methods:

The plant material was collected as gift sample of Micobiotech limited Gujrat India as dried samples and it was coarsely grounded in to fine powder using electrical blender before extraction. The material was then passed through #120 mesh to obtain uniform size powder. The powdered plant material was successively extracted separately with solvents of increasing polarity like petroleum ether, chloroform and methanol through continuous extraction process by Soxhlet extractor. Before extraction with each solvent, the powdered material, as well as each extract (residue), was air-dried below 50 °C, and the completion of extraction was indicated by taking a sample out of siphon tube on TLC plate and placing it in iodine chamber. The extract was sifted through a Buchner funnel with Whatman No. 1 filter paper. This was repeated three times for the complete extraction of compounds and all three solvent extracts were pooled. The extracts were concentrated under the vacuum, and dried along solvent was distilled off. By the distillation process the extract was concentrated to a semisolid consistency. To evaporate the solvent the residue of the extract was kept in a wide mouth beaker at room temperature for about 8 to 10 hrs. The crude extract was used for the phytochemical studies [8].

Qualitative Phytochemical Analysis of different extracts of *Sargassum Wightii*.

The different qualitative analysis of Phytochemicals of S wightii were assessed by standard method and it was carried out to know the presence of compounds like alkaloids, glycosides, steroids, saponins, tannins, flavonoids , proteins and phenolic compounds etc. General reactions in these analyses revealed the presence or absence of these compounds in the seaweed extracts tested[9,10,11,12].

Test for Alkaloids

All the extract of S. wightii were filtered separately and were dissolved with 2N hydrochloric acid and separated into three equal portion for each extracts, one for Mayer's tests, second one for Dragondroff's reagent and last one for Wagner's reagents. The emergence of creamy white precipitate, orange precipitate and reddish brown precipitate respectively indicated the presence of alkaloids in given sample.

Tests for Glycosides

About 0.5 gm of each extract was taken in a test tube and 1 ml of glacial acetic acid was added followed by one drop of ferric chloride solution, then 1 ml of concentrated sulphuric acid was added at side and the formation of brown ring at the junction indicated the presence deoxy sugar that was the characteristic presence of glycosides.

Tests for Phenols

About 2 ml each S wightii extracts were taken, 2 ml of distilled water followed by few drops of freshly prepared 10% of Ferric chloride solution was added respectively. Formation of blue black colour indicated the presence of phenols in the algal extract.

Tests for Flavonoids

With 2 ml of extract was added to 1.5 ml of 50% methanol solution. The solution was warmed and added magnesium metal. In continuation added a few drops of conc. Hydrochloric acid and the red color were formed which showed the presence of flavonoids and the orange color indicated the presence of flavones.

Tests for Tannins

About 0.5 g of *S wightii* extract was taken and added to a 10 ml of water kept in the test tube and filtered. Added a few drops of freshly prepared 5% ferric chloride and observed for brownish green or blue-black coloration. A brownish green color was formed which indicate the presence of tannins.

Test for Saponins

A 2ml of the extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins[13].

Test for Steroids

2 ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H_2SO_4. The colour changed from violet to blue or green indicate the presence of steroid[14].

Test for Proteins

To a 2ml of methanolic extract, 1ml of 40% NaOH solution was added and added 2 drops of 1% CuSO4 solution. The presence of a peptide linkage of the molecule was indicated by the violet color which shows the presence of protein.

Quantitative analysis of phytochemical substances in *Sargassum wightii* extracts

Estimation of phenols

The total phenols in different S wightii extracts were determined by a modified method as described by Siddiqu N *et al*[15]. The assay involved gallic acid as the standard. The Gallic acid in the range of 20 -200 mg /l was used as standard for the construction of the calibration curve. 1 ml of 10% Folin Ciocalteu reagent was added to 20ml of different extract and the standard. The samples were mixed well and incubated for 5 to 10 min followed by addition 0.7 ml of 10% Na_2CO_3. Again the mixed sample was incubated for 2 hours and absorbance has been taken at 765nm using UV/VISIBLE Spectrophotometer (Schimadzu, Japan) against blank, i.e., distilled water. All the values were taken thrice and the estimation of phenol was done by Gallic acid equivalent/ (GAE/g) of dried weight.

Estimation of Flavoniods

Estimation of flavonoids was done by Aluminium Chloride colorimetric method[16] and Rutin in the range of 20-200mg/l had been used for the calibration curve. 0.5 ml of 2% aluminium chloride was mixed with equal volume of (different) extract of S wightii then incubated for 1 hour at room temperature and the absorbance of the mixture was measured at 415 nm using UV/Visible Spectrophotometer. Estimation of the total flavonoids was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg Rutin equivalents (RUE/g) dry weight.

Estimation of Tannins

Estimation of tannins was done by using (method) Catechin as standard; 20-200mg/l of Catechin had been used for the calibration curve. 5ml of extract mixed with 1.5 ml of 40% vanillin followed by addition of 0.75ml of HCl. The solution was mixed well and incubated for 20 min at room temperature then the absorbance of solution mixture were taken at 500 nm using UV/VISIBLE Spectrophotometer. Estimation of tannins content was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg catechin equivalents /g (CAE /g) dried weight[17].

Results and Discussion:

Qualitative analysis of phytochemical substances in Sargassum Wightti extracts

In the present study, preliminary phytochemical screening of 8 different chemical compounds (alkaloids, steroids, tannins, saponins, flavonoids, phenols, protiens and glycosides) were performed with petroleum ether, chloroform and methanol extracts of *Sargassum wightti*. The patterns of composition differed considerably in their varied degree. The results of phytochemical evaluation were shown in Table 1. Saponins did not show any positive result for their presence in any of the extracts.

Among the three different extracts, methanol extract showed the presence of maximum number of seven compounds. Next to that, chloroform extract showed five compounds. Petroleum ether extracts showed only three compounds with very less intensity. Methanol extract showed the presence of alkaloids, steroids, flavonoids, phenols, glycosides, tannins and proteins. Chloroform extract showed the presence of phenols, tannins, flavonoids, steroids and proteins. Petroleum ether extract showed the mild presence of flavonoids, tannins, and steroids. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction.

Alkaloids have a wide range of pharmacological activities including antimalarial, anticancer , cholinomimetic ,vasodilatory , antiarrhythmic , analgesic , antibacterial and antihyperglycemic activities [18,19]. Alkaloids have cytotoxic activity that is due to the presence of microtubule interfering agents that can bind to beta tubulin, thus inhibiting the formation of the mitotic spindle fibre required for cell division [20]. Steroids of seaweeds are known to be important for immunomodulators, antiarthritic agent, antioxidant, radical scavenging agents, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine, cosmetics and skin conditioning agents[21]. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent. Tannins is also being used in the process of tanning leather[22]. Flavonoids showed its presence in all tested extracts. Flavonoids have antimicrobial, antiviral, antioxidant and spasmolytic activity[23]. Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. Phenols also showed its presence in all extracts of *Sargassum wightti*. In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs[24]. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions[25]. Saponins did not show any positive result in any extract of *Sargassum Wightti.*

Sl. No	Phytochemical Parameters	Petroleum Ether	Chloroform	Methanol
1	Alkaloids	--	--	+
2	Glycosides	--	--	+
3	Phenols	--	+	++
4	Flavonoids	+	++	++
5	Tannins	+	+	++
6	Saponins	--	--	--
7	Steroids	+	++	++
8	Proteins	--	+	+

++: intensely present, +: Present, - : Absent

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Sargassum Wightti*

Quantitative analysis of phytochemical substances of Sargassum Wightti extracts

Phenolics, flavonoids and tannins contents of *Sargassum Wightti* were estimated quantitatively varied according to solvents used in extraction processes[26]. The highest total phenolics (2.74 ± 0.17 mg GAE/g dry wt) and tannins (2.65 ± 0.43 mg CAE /g dry wt) was recorded in methanol extract, while the highest total flavonoids (1.97 ± 0.03 mg RUE/g dry wt) was recorded in chloroform extract of *Sargassum wightti* (Table-2).

Solvents	Total Phenolics (mg GAE/g dry wt)	Total Flavonoids (mg RUE/g dry wt)	Total Tannins (mg CAE/g dry wt)
Petroleuum ether	--	0.81±0.05	1.75±0.04
Chloroform	1.12±0.15	1.97±0.03	1.52±0.47
Methanol	2.74±0.17	1.62±0.07	2.65±0.43

Table 2: Quantitative analyses of phytochemical substances present in different extracts of *Sargassum Wightti*

Values are means of three analyses of the extract ± standard deviation (n=3) GAE: Gallic acid equivalent, RUE: Rutin equivalent, CAE: Catechin equivalent.

Conclusion:

In the present study an attempt has been made to carryout preliminary phytochemical screening of the different extracts of *Sargassum Wightti* and shown the presence of alkaloids, flavonoids, phenolic compounds, steroids, tannins, glycosides and proteins in the extracts. These preliminary phytochemical screenings shown the presence of active compounds those have pharmacological action. Extraction solvents have an effect on yield of total phenolics, total flavonoids and total tannins from *Sargassum Wightti*. Thus these seaweeds could be collected and utilized effectively in product preparation for the beneficial of mankind.

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References

[1] Willam CV: Trease and evans pharmacognosy. Saunders elsvier publishers,16th edition 2009.
[2] Antonisamy JM, Essakimuthu P, Narayanan J, Anantham B, Tharmaraj RJ, Arumugam S: Phytochemical characterization of brown seaweed Sargassum wightii. Asian Pacific Journal of Tropical Disease 2012 ;109-113
[3] Faulkner DJ: Highlights of marine natural products chemistry (1972-1999). Natural Product Reports 2002; 17: 1-6.
[4] Thiruchelvi R, Jayashree P, Hemashree T, Hemasudha TS, Balashanmugum P: Preliminary Phytochemical analysis of the crude extract of marine red and brown seaweeds. Research Journal of pharmacy and Technology 2018;1-4.
[5] Deyab M, Elkatony T and Ward F: Qualitative and Quantitative Analysis of Phytochemical Studies on Brown Seaweed, Dictyota dichotoma. International Journal of Engineering Development and Research 2016;4(2): .674-678.
[6] Chandini SK, Ganesan P, Bhaskar N: In vitro antioxidant activities of three selected brown seaweeds of India. Food Chemistry 2008; 107: 707-713.
[7] Ganesan P, Chandini SK, Bhaskar N: Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresource Technology 2008;99: 2717-2723.
[8] Nagulwar VP and Deshpande SA: Phytochemical screening and evaluation of pharmacological activities of *Buchanania lanzan* spreng leaves. International Journal of Pharmaceutical Sciences and Research 2020; 11(1): 156-162.
[9] Arokiyaraj K, Perinbam P, Agastian R and Mohan K: Phytochemical analysis and antibacterial activity of *Vitex agnuscastus*. International Journal of Green Pharmacy2009; 34:162-164.
[10] Titto RJ: Phenol constituents in the leaves of northern Willows methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry 1985; 33:213–217.
[11] Janarthanan M and Kumar MS: Qualitative and quantitative analysis of phytochemical studies on selected seaweeds *Acanthopora spicifera* and *Sargassum wightii*. International Journal of Engineering Research and Development 2013; 7: 11-15.
[12] Kokate C K: Practical pharmacognosy, Vallabhprakashan, 4th edition, 1994.
[13] Harbourne J.B: Phytochemical methods: A guide to modern technique of plant analysis. Chapman and Hall Ltd, 2nd edition 1998.
[14] Hossain MA, Salim KA, Raqmi AL, ALMijizy ZH, Weli AM, and Riyami QA: Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pacific Journal of Tropical Biomedicine 2013; 3(9): 705–710.
[15] Siddiqu N, Rauf A, Latif A, Mahmood Z: Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). Journal of Taibah University Medical Sciences 2017:4: 360-363.
[16] Eldalway R: Quantitative estimation of rutin in RUE (Ruta Graveolens L) cultivated in Iraq with the evaluation of its antntioxidant activity. *Asian Journal of Pharmaceutical and Clinical Research*, Vol. 10, no. 2, Feb. 2017, pp. 353-5,
[17] Medini F, Fellah H, Ksouri R, and Abdelly C: Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. Journal of Taibah University for Science2014; 8(3):216-224
[18] Guven KC and Sunam BG: Hordenine from the alga *Phyllophora nervosa*. Phytochemistry 1970; 9(8):1893-1997.
[19] Kasım C G, Percot A and Sezik E: Alkaloids in Marine Algae. Marine Drugs 2010; 8: 269-284
[20] Solanki R, Khanna M and Lal R: Bioactive compounds from marine actinomycetes. Indian Journal of Microbiology 2008;48: 410–431.
[21] Okwu DE: A improving Nutritive value of cassava tapiocmeal with local spices. Journal of Nutraceutical Functional and Medical Foods 2001; 3: 43-51.
[22] Kolodziej H and Kiderlen AF: Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. Phytochemistry 2005; 66(17): 2056-2071.
[23] Farasat M, Nejad AK, Nabavi MB, and Namjooyan F:Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from Northern Coasts of the Persian Gulf. Iranian Journal of Pharmaceutical Research 2014;13(1): 163–170.
[24] Chakraborty K, Praveen NK, Vijayan KK and Rao GS: Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to Turbinaria spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine 2013; 3(1): 8-16.
[25] Aliyu AB, Musa AM, Sallau MS and Oyewale OA: Proximate composition, mineral elements and anti-nutritional factors of Anisopus mannii N.E.Br. (Asclepiadaceae). Trends in Applied Sciences Research 2009; 4(1):68-72.
[26] Iloki Assanga SB, Lewis-Lujan LM, Lara-Espinoza CL, Gil-Salido AA, Angulo DF, Pino JR and Haines DD: Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Research Notes 2015; 8: 396-402.