Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Short Communication

PHYTOCHEMICAL COMPOSITION OF *SARGASSUM POLYCYSTUM* C. AGARDH AND *SARGASSUM DUPLICATUM* J. AGARDH

ASHA KANIMOZHI S, JOHNSON M*, RENISHEYA JOY JEBA MALAR T

Centre for Plant Biotechnology, PG and Research Department of Botany, St. Xavier's College (Autonomous), Palyamkottai, Tamil Nadu, India
Email: ptcjohnson@gmail.com

Received: 31 Mar 2015 Revised and Accepted: 26 Jun 2015

ABSTRACT

Objective: The present study was aimed to reveal the phytochemical composition of *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh from Manapad, Thoothukudi district, Tamil Nadu, India.

Methods: Seaweeds *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh were collected from Manapad, Thoothukudi district, Tamil Nadu, India by hand picking method. The dried and powdered materials (10 g) of *S. polycystum* and *S. duplicatum* were extracted with 60 ml of solvents viz., petroleum ether, chloroform, acetone, Methanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. The different extracts were tested for steroids, terpenoids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, cardiac glycosides, anthraquinone and sterol. Phytochemical screening of extracts was carried out according to the standard method. To know the extractive values and physicochemical characters of *S. polycystum* and *S. duplicatum*, the ash and fluorescence analysis was determined by standard method.

Results: Among the various tested extracts, methanolic extracts of *S. polycystum* showed the presence of the maximum of seven metabolites out of ten metabolites examined. Next to that chloroform and acetone extracts of *S. polycystum* displayed the occurrence of four metabolites. Petroleum ether extract of *S. polycystum* demonstrated the presence of three metabolites. Aqueous extracts of *S. polycystum* showed the occurrence of only two metabolites. The methanolic and chloroform extracts of *S. duplicatum* showed their presence of maximum of five metabolites out of ten metabolites examined. Next to that acetone extract of *S. duplicatum* displayed four metabolites. Petroleum ether extract of *S. duplicatum* demonstrated the occurrence of three metabolites in the crude extracts. Aqueous extract of *S. duplicatum* displayed the presence of two metabolites. The characteristic fluorescent properties or colours emitted by the powdered thallus of *S. polycystum* and *S. duplicatum* before and after treating with various extracts were recorded.

Conclusion: To strengthen the global scientific effort, in the present study the phyto-constituents presence in *S. polycystum* and *S. duplicatum* are documented.

Keywords: *Sargassum*, Phytochemistry, Chemical diversity.

Marine environment is a rich source of biological and chemical diversity. The diversity has been a unique source of chemical compounds of potential for pharmaceuticals, cosmetics, dietary supplements and agrochemicals [1]. In folk medicine, seaweeds have been used for a variety of remedial purposes, such as in eczema, gallstone, renal trouble, scabies, Psoriasis, asthma, arteriosclerosis, heart disease, ulcers and cancer [2]. Seaweeds are considered as a rich source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [3]. Red and brown seaweeds are rich sources of bioactive secondary metabolites. Numerous studies have focused on their nutraceutical and pharmaceutical properties [4-6]. *Sargassum*, a genus of brown seaweed, commonly known as gulf-weed or sea holly belonging to family Sargassaceae, order Fucales, subclass Cyclosporeae, and class Phaeophyceae, contains approximately 400 species [7, 8].

For nearly 2000 years *Sargassum* spp., brown seaweed, has been used in Traditional Chinese Medicine (TCM) to treat a variety of diseases including thyroid disease (e. g. goitre). *Sargassum* has been used traditionally for treating scrofula, goiter, tumor, edema, testicular pain and swelling [7]. The therapeutic effects of *Sargassum* spp. are scientifically plausible and may be explained partially by key *in vivo* and *in vitro* pharmacological activities of *Sargassum*, such as anticancer, anti-inflammatory, antifouling, antibacterial [9-13], antiviral [8, 14, 15], Hepatoprotective [2], Larvicidal [17]; neuroprotective [18] and Anti HIV [19].

Sargassum species are found throughout tropical and subtropical areas of the world and are reported to produce metabolites of structural classes such as terpenoids, polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides etc., which possesses several therapeutic activities. As it possesses many pharmacological properties, it has been considered as a medicinal food of the twenty-first century, and research is being carried out on it to reveal its other pharmacological properties. The metabolites meroterpenoids, phlorotanins and fucoidans are isolated from *Sargassum*. The contribution of iodine in *Sargassum* for treating thyroid related diseases seems to have been over estimated. *Sargassum* species are used as fodder and fertilizer in China and many parts of Asia [20]. *Sargassum* forms about 10% of the average diet in Japan where tender parts of the plant are eaten raw as salad or cooked with coconut milk. Also in Bermuda, indigenes spread out salt free *Sargassum* clumps as fertilizer around the base of banana.

Algin, a carbohydrate found in *Sargassum* is extracted for use in textile, paper and pharmaceutical industries [21]. *Sargassum* biomass is used as a potential renewable energy resource such as biogas [22, 23]. Bhaigyabati and Usha [24] confirmed the presence of phytoconstituents such as alkaloids, amino acids, anthraquinones, carbohydrates, phenolic compounds, flavonoids, terpenoids, steroids and tannins in *Sargassum wightii* from Kanyakumari. In addition they identified the antioxidant potentials of *S. wightii*.

Sumithra and Arunachalam [25] studied the phytochemical composition of *Sargassum ilicifolium.* Madhan Chakkaravarthy and Kumar [26] revealed the HPTLC Finger print profile of steroid, flavonoid *Sargassum wightii* from Gulf of Mannar and estimated antiradical activity. Arun Kumar *et al.* [27] synthesised silver nanoparticles from *Sargassum polyphyllum* and evaluated their antibacterial potentials. Bhaigyabathi *et al.* [28] studied the phytochemical composition of *Sargassum muticum* and evaluated their antioxidant activity. Farook Basha and Muthukumar [29] isolated steroid from *Sargassum ilicifolium* evaluated their anitgiotension properties. Johnson *et al.* [30] and Devi *et al.* [31]

explored the pharmacognostical and phytochemical properties of *Sargassum wightii.* With this knowledge the present study was aimed to reveal the phytochemical composition of *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh from Manapad, Thoothukudi district, Tamil Nadu, India.

Seaweeds *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh were collected from Manapad, Thoothukudi district, Tamil Nadu, India by hand picking method. The collected samples were authenticated by Dr. D. Patric Raja, Associate Professor of Botany, St. Xavier's Collge (Autonomous), Palayamkottai. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. It was then thoroughly washed with tap water followed by distilled water to remove the unwanted debries. Seaweeds were blotted on the blotting paper and spread out at room temperature in the shade for 20 days. The shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further analysis.

The dried and powdered materials (10 g) of *S. polycystum* and *S. duplicatum* were extracted with 60 ml of solvents viz., petroleum ether, chloroform, acetone, and methanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

The different extracts were tested for steroids, terpenoids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, cardiac glycosides, anthraquinone and sterol. Phytochemical screening of extracts was carried out according to the standard method described by Harborne [32].

To know the extractive values and Physico chemical characters of *S. polycystum* and *S. duplicatum*, the ash and fluorescence analysis was determined by standard method. The different extracts of *S. polycystum* and *S. duplicatum* were examined under visible and UV light. The powdered materials were also treated with various reagents such as, H_2SO_4, HCl, NaOH, CH_3COOH, $FeCl_3$ and changes in colour were recorded in visible and UV light.

Among the various tested extracts, methanolic extracts of *S. polycystum* showed the presence of the maximum of seven metabolites out of ten metabolites examined. Next to that chloroform and acetone extracts of *S. polycystum* displayed the occurrence of four metabolites. Petroleum ether extract of *S. polycystum* demonstrated the presence of three metabolites. Aqueous extracts of *S. polycystum* showed the occurrence of only two metabolites. The steroids and sterol showed their existence in methanolic, petroleum ether, acetone and chloroform extracts of *S. polycystum*. Cardiac glycosides showed its presence in methanolic, petroleum ether and chloroform extracts of *S. polycystum*. Phenolic groups showed its occurrence in the methanolic, chloroform and aqueous extract of *S. polycystum*. Flavonoids showed its presence in the methanolic and acetone extract of *S. polycystum*. Saponin displayed its occurrence in the methanolic and acetone extract of *S. polycystum*. Alkaloids showed its presence only in methanolic extract of *S. polycystum*. Subsequently tannin was only in the aqueous extract of *S. polycystum*. Anthraquinone and terpenoids failed to show their presence in any of the tested extracts of *S. polycystum* (table 1). Similarly, the methanolic and chloroform extracts of *S. duplicatum* showed their presence of the maximum of five metabolites out of ten metabolites examined. Next to that acetone extract of *S. duplicatum* displayed four metabolites. Petroleum ether extract of *S. duplicatum* demonstrated the occurrence of three metabolites in the crude extracts. Aqueous extract of *S. duplicatum* displayed the presence of two metabolites. Steroids, phenolic groups and sterol showed their presence in methanolic, petroleum ether, acetone and chloroform extracts of *S. duplicatum*. Flavonoids showed its occurrence in the methanolic, chloroform and aqueous extract of *S. duplicatum*. Saponin displayed its occurrence in the methanolic, chloroform and acetone extract of *S. duplicatum*. Similarily, tannin showed its presence only in the aqueous extract of *S. polycystum*. Anthraquinone, cardiac glycosides, alkaloids and terpenoids failed to show their presence in any of the tested extracts of *S. duplicatum* (table 1).

The fluoresence analysis of the selected seaweed extracts *S. polycystum* and *S. duplicatum* were recorded and displayed in fig. 1. The characteristic fluorescent properties or colours emitted by the powdered thallus of *S. polycystum* and *S. duplicatum* before and after treating with various extracts were recorded.

Table 1: Phytochemical analysis of different extracts of *S. polycystum* and *S. duplicatum*

Metabolites	***S. polycystum***					***S. duplicatum***				
	P	**C**	**A**	**M**	**AQ**	**P**	**C**	**A**	**M**	**AQ**
Steroids	+	+	+	+	-	+	+	+	+	-
Alkaloids	-	-	-	+	-	-	-	-	-	-
Phenolic groups	-	+	-	+	+	+	+	+	+	-
Cardiac glycosides	+	+	-	+	-	-	-	-	-	-
Flavonoids	-	-	+	+	-	-	+	-	+	+
Saponins	-	-	+	+	-	-	+	+	+	-
Tannins	-	-	-	-	+	-	-	-	-	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-	-
Sterol	+	+	+	+	-	+	+	+	+	-
Total	3	4	4	7	2	3	5	4	5	2

Note: P-Petroleum Ether; C-Chloroform; A-acetone; M–methanol; Aq–aqueous

Seaweeds refer to any large marine benthic algae that are multicellular, macrothallic, and thus differentiated from most algae that are of microscopic size. These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial [33-35]. Seaweeds are the extraordinary sustainable resources which have been used as a source of food, feed and medicine [36]. *Sargassum, Padina, Dictyota* and *Gracilaria* sps can be used as fertilizers, food additives and animal feed [37, 38]. Seaweeds offer a wide range of therapeutic possibilities both internally and externally.

They are extensive profile source of secondary metabolites. More than 2400 seaweed secondary metabolites have been isolated from marine algae [39]. Although a majority of these (about 60%) are terpenes, but some fatty acids are also common (20%) with nitrogenous compounds [40], These metabolites are mainly terpenes, aceto genins alkaloids and polyphenolics, with many of these compounds being halogenated [41].

Alkaloids are commonly found to have antimicrobial properties [42] against both Gram-positive and Gram-negative bacteria [43]. The results for phytochemical screening of *S. polycystum* and *S. duplicatum* revealed the alkaloids presence only in the methanolic extract of *S. polycystum* and absent in the *S. duplicatum*. Seaweeds extracts are considered to be a rich source of phenolic compounds [44, 45]. The results of the present study confirmed the phenolic compounds presence in all the tested extracts of studied *Sargassum* species except petroleum ether and acetone extract of *S. polycystum* and aqueous extract of *S. duplicatum*. Phenolic compounds are commonly found in plants, including seaweeds and have been

reported to have a wide range of biological activities including antioxidant properties [46-48]. The Folin-Ciocalteu method was applied to study the total phenolic content of the seaweeds. Folin-Ciocalteu reagent determines total phenols, producing blue colour by reducing yellow heteropolyphosphomolybate-tungstate anions [49]. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae [50]. The total phenolic content results of *S. marginatum* and *P. gymnosperma* obtained in this study were higher than some reports for other brown seaweeds. Chandini *et al.* [37] reported methanolic extract of contain 24.61 and 49.16 mg GAE/g phenolic content. Wang *et al.* [48] reported the total phenolic content in different Icelandic seaweeds ranging from 4 to 242 mg PGE/g extract; of which, *P. palmata* showed the lowest total phenolic content. Similar to the previous observation on the phenolic content, in the present study also *S. polycystum* and *S. duplicatum* showed the phenolic presence in the methanolic extracts.

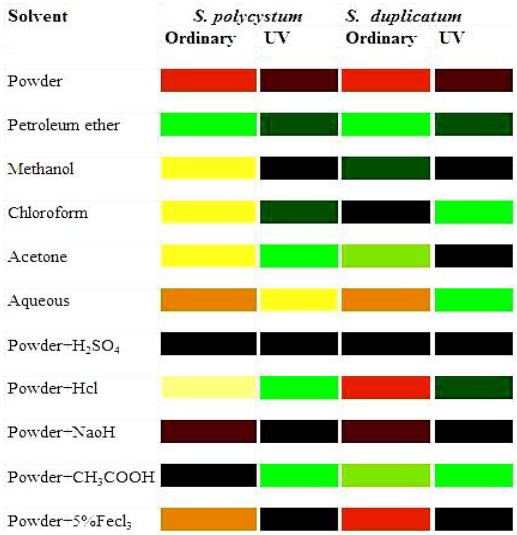

Fig. 1: Fluorescence analysis of *S. polycystum* and *S. duplicatum*

Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolyzable compounds. Hydrolyzable tannins are gallic acid which easily hydrolyzes in acidic media, and condensed tannins are polymeric flavonoids [51]. Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants [52, 53]. In contrast to terrestrial tannins, phlorotannins are tannin compounds which have been found only in marine algae. Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga [54-56]. Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton [57]. Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings [58]. Many tannin-containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote. Tannins has been found to have antiviral, antibacterial, antiparasitic effects,anti-inflammatory, anti ulcer and antioxidant property for possible therapeutic applications [59-61]. The present study results confirm the tannin presence only in aqueous extract of *S. polycystum* and *S. duplicatum*.

Flavonoids, the largest groups of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties. Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and iso flavonoids [62]. Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation [63, 64]. Many marine plants, including seaweeds, often carry significantly less macro and micro epibionts on their thalli compared to co-occurring biofilms on inanimate substrata [65, 66]. Therefore it has been assumed that seaweeds defend themselves against bacterial fouling by production of secondary metabolites that prevent attachment and growth of bacterial colonizers. Recently, consumers are demanding foods which are fresh, natural and minimally processed along with the requirement for enhanced safety and quality. This perspective has put pressure on the food industry for progressive removal of chemical preservatives, and has fuelled research into alternative natural antimicrobials. Plant products with antimicrobial properties have obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth. In the present study, the presence of flavonoid was observed in acetone and methanolic extracts of *Sargassum* species. In *S. duplicatum*, flavonoid showed its presence in all the extracts except in petroleum ether and acetone extract.

Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce an inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutraceuticals. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti inflammatory, anti feedent and hemolytic effects [66, 67]. These observations cited on phytochemical compounds support our findings on the usefulness of seaweeds in traditional medicament. In the present investigation saponin also showed its presence in *S. polycytum* and *S. duplicatum*.

The plants known as medicinal, are rich in secondary metabolites which includes alkaloids, glycosides, flavonoids, insecticides, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of plants across the world. In the present investigation also, two seaweeds have been selected from India for phyto chemical screening on the basis of traditional uses. The present phyto chemical study revealed the presence of phenols, alkaloids, tannins, steroids, glycosides, saponin and flavonoids in *S. polycystum* and *S. duplicatum* with varied degree. Many workers revealed that the crude extracts of Indian seaweeds are active

against Gram-positive bacteria [68]. The antibacterial and pharmacological activity of the *Sargassum* species may be due to one/more group of above phyto-constituents.

The fluorescence analysis is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples. In the present study, also we developed the fluorescence analysis for crude powder and various extracts of *S. polycytum* and *S. duplicatum* and represented in varied colours. Recently, chemists worldwide have paid attention to the potential of marine organisms as alternative sources for the isolation of novel metabolites with interesting biological and pharmaceutical properties. To strengthen the global scientific effort, in the present study the phyto-constituents presence in *S. polycystum* and *S. duplicatum* are documented.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Chau Van M, Phan Van K, Ngyen Hai D. Marine natural products and their potential application in the future. AJST 2005;22(4):297-11.
2. Balaji raghavendran H, Sathivel A, Devaki T. Defensive nature of *Sargassum polycystum* (Brown alga) against acetaminophen-induced toxic hepatitis in rats: Role of drug metabolizing microsomal enzyme system, tumor necrosis factor-α and fate of liver cell structural integrity. World J Gastroenterol 2006;12(24):3829-4.
3. Rajasulochana P, Dhamotharan R, Krishnamoorthy P, Murugesan S. Antibacterial activity of the extracts of marine red and brown algae. J Am Sci 2009;5(3):20-5.
4. Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2007;24:31-86.
5. Cabrita L, Quintela JM, Vilalta R. *In vitro* activities of three selected brown seaweeds of India. An Quim Ser C 2010;8:113–5.
6. Narisnh L, Archana N, Werner EG. Muller. Marine natural products on drug discovery. Nat Prod Radiance 2005;4(6):28-48.
7. Kandale A, Meena AK, Rao MM, Panda P, Mangal AK, Reddy G, *et al.* Marine algae: An introduction, food value and medicinal uses. J Pharm Res 2011;4(1):219-21.
8. Bazes A, Silkina A, Douzenel P, Fay F, Kervarec N, Morin D, *et al.* Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. J Appl Phycol 2009;21(4):395.
9. Arputha Bibiana M, Nithya K, Manikandan MS, Selvamani P, Latha S. Antimicrobial evaluation of the organic extracts of *Sargassum wightii* (brown algae) and *Kappaphycus alwarezii* (red algae) collected from the coast of Meemesal, Tamilnadu. IJPCBS 2012;2(4):439-6.
10. Mansuya P, Aruna P, Sridhar S, Suresh Kumar J, Babu S. Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar Region. J Exp Sci 2010;1(8):23-6.
11. Sherwani SK, Nazim K, Khan TM, Ahmed M, Malik MW, Noor AA, *et al.* Phytochemical and antibacterial screening of crude extract of *Sargassum tenerrimum* J. Agardh against potential human pathogens. Fuuast J Biol 2012;2(2):65-8.
12. Karthick N, Murugalakshmi Kumari R. Evaluation of phytochemical analysis and antimicrobial activity of different solvent extracts of *Sargassum wightii*. Int J Res Plant Sci 2014;4(4):96-9.
13. Sangeetha S, Dhayanithi NB, Sivakumar N. Antibacterial activity of *Sargassum longifolium* and *Gracilaria corticata* from Gulf of Mannar against selected common shrimp pathogens. Int J Pharm Biol Sci 2014;5(2):76–2.
14. Yangthong M, Towatana NH, Phromkunthong W. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Foods Hum Nutr 2009;64(3):218-3.
15. Liu L, Heinrich M, Myres S, Dworjanyn SA. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in traditional chinese medicine: a phytochemical and pharmacological review. J Ethnopharmacol 2012; 142(3):595.
16. Achary A, Muthalagu K, Saravana Guru M. Identification of Phytochemicals from *Sargassum* wightii against *Aedes aegypti*. Int J Pharm Sci Rev Res 2014;29(1):314-9.
17. Lee SG, Kang H. Neuroprotective Effect of *Sargassum thunbergii* (Mertens ex Roth) kuntze in activated murine microglial cells. Trop J Pharm Res 2015;14(2):235-40.
18. Subramaniam D, Menon T, Elizabeth HL, Swaminathan S. Anti-HIV-1 activity of *Sargassum swartzii* a marine brown alga. BMC Infect Dis 2014;14(Suppl 3):E43.
19. Round FE. The Biology of the Algae. 2nd ed. London: Edward Arnold Publishers Ltd; 1973.
20. Chennubhotla VSK, Kaliaperumal N, Kalimuthu S. Seaweed recipes and other practical uses of seaweeds. Sea Food Exp J 1981;13:9-16.
21. Wang S, Jiang XM, Han XX, Liu JG. Combustion characteristics of seaweeds biomass. 1. Combustion characteristics of *Enteromorpha clathrata* and *Sargassum natans*. Energy Fuels 2009;23:5173-8.
22. Yokoyama S, Jonouchi K, Imou K. Energy production from marine biomass: fuel cell power generation driven by methane produced from seaweed. World Acad Sci Eng Technol 2007;28:320-3.
23. Bhaigyabati T, Usha K. Preliminary phytochemical screening and antioxidant activity of various extracts of *Sargassum wightii* Greville. Int J Universal Pharm Biol Sci 2013;2(6):60-9.
24. Sumithra M, Arunachalam G. Pharmacognostical study and phytochemical evaluation of *Sargassum ilicifolium* (Turner) C. Agardh. Int J Pharm Tech Res 2014;6(7):2022-7.
25. Madhan Chakkaravarthy V, Kumar V. HPTLC Finger print analysis of steroid, Flavonoid and antiradical activity in *Sargassum wightii* from Gulf of Mannar. Res J Pharmacogn Phytochem 2011;3(2):72-4.
26. Arunkumar M, Suhashini K, Mahesh N, Ravikumar R. Quorum quenching and antibacterial activity of silver nanoparticles synthesized from *Sargassum polyphyllum*. Bangladesh J Pharmacol 2014;9:54-9.
27. Bhaigyabathi T, Kirthika T, Shiny K, Usha K. Phytochemical screening and antioxidant activity of various extracts of *Sargassum muticum*. IJPRD 2011;3(10):25-30.
28. Farook Basha S, Muthukumar C. Preliminary phytochemical screening and invitro angiotension activity of bioactive compound-steroid isolated from *Sargassum ilicifolium*. Int J Pharm Pharm Sci 2014;6(Suppl 2):299-301.
29. Johnson M, Petchiammal E, Janakiraman N, Babu A, Renisheya Joy Jeba Malar T, Sivaraman A. Phytochemical characterization of brown seaweed *Sargassum wightii*. Asian Pac J Trop Dis 2012;2(Suppl 1):S109-13.
30. Devi JAI, Sathiya Balan G, Periyanayaga K. Pharmacognostical study and phytochemical evaluation of brown seaweed *Sargassum wightii*. J Coastal Life Med 2013;1(3):199-4.
31. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. New York: Chapman and Hall; 1998.
32. Faulkner DJ. Highlights of marine natural products chemistry. Nat Prod Rep 2000a;17:1–6.
33. Faulkner DJ. Marine pharmacology. Antonie Van Leeuwenhoek 2000b;77:135–45.
34. Schwartsmann G, Rocha DA, Berlinck AB, Jimeno J. Marine organisms as a source of new anticancer agents. Lancet Oncol 2001;2:221–5.
35. Dhargalkar VK, Neelam P. Seaweed: Promising plant of the millennium. Sci Culture 2005;71:60-6.
36. Chandini SK, Ganesan P, Bhaskar N. *In vitro* activities of three selected brown seaweeds of India. Food Chem 2008;107:707-13.
37. Dawczynski C, Schubert R, Jahreis G. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem 2007;103:891-9.
38. Fitton JH. Antiviral properties of marine algae. In: Critchley AT, Ohno M, Largo DB. Editors. World Seaweed Resources. Workingham, UK: ETI Information Services; 2006. p. 7.
39. Van Alstyne KL, Paul VJ. The role of secondary metabolites in marine ecological interactions. Proc Sixth Int Coral Reef Symphosium 1988;1:175-86.

40. Watson SB, Cruz-Rivera E. Algal chemical ecology: an introduction to the special issue. Phycologia 2003;42:319-23.
41. Omulokoli E, Khan B. Chhabra. Antiplasmodial activity of four Kenyan medicinal plants. J Ethnopharmacol 1997;56:133-7.
42. Cowan MM. Plants products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
43. Athukorala Y, Lee KW, Shahidi F, Heu MS, Kim HT, Lee JS, *et al.* Antioxidant efficacy of extracts of an edible red alga (*Grateloupia wlicina*) in linoleic acid and Wsh Oil. J Food Lipid 2003;10:313-27.
44. Heo SJ, Park EJ, Lee KW, Jeon YJ. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 2005;96:1613-23.
45. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chem 2006;95:37-43.
46. Kuda T, Kunii T, Goto H, Suzuki T, Yano T. Varieties of antioxidant and antibacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* products harvested and processed in the Noto peninsula, Japan. Food Chem 2007;103:900-5.
47. Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem 2009;116:240-8.
48. Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food Chem Toxicol 2006;44:1065-74.
49. Nagai T, Yukimoto T. Marine natural products on drug discovery. Food Chem 2003;81:327-32.
50. Huang J, Liu Y, Wang X. Selective adsorption of tannin from favonoids by organically attapulgite clay. J Hazard Mater 2008;160:382-7.
51. Haslam E. Plant Polyphenols: Vegetable Tannins Revisited. Cambridge UK: Cambridge University Press; 1989.
52. Waterman PG, Mole S. Analysis of Phenolic Plant Metabolites. Oxford, UK: Blackwell Scientifc Publications; 1994.
53. Ragan MA, Glombitza KW. Phlorotannins, brown algal polyphenols. In: Hellebustad JA, Craigie JS. editors. Handbook of Phycological Methods. Cambridge: Cambridge University Press; 1986.
54. Sarah JW, Bloor SJ, Hemmingson JA, Furneaux RH, Nelson WA. The presence of gigartinine in a New Zealand *Gracilaria* species. J Appl Phycol 2001;13:409–13.
55. Hemat RAS. Fat and muscle dysfunction. In: Hemat RAS. editor. Andropathy. Dublin, Ireland; 2007. p. 83-5.
56. Lü L, Liu SW, Jiang SB, Wu SG. Tannin inhibits HIV-1 entry by targeting gp 41. Acta Pharmacol Sin 2004;25(2):213–8.
57. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother 2001; 48(4):487-91.
58. Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. Phytochem 2005; 66(17):2056-71.
59. Ndhlala AR, Kasiyamhuru A, Mupure C, Chitindingu K, Benhura MA, Muchuweti M. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chem 2007;103:82-7.
60. Herrmann K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in food. CRC Crit Rev Food Sci Nutr 1989;28:315-47.
61. Wallace G, Fry SC. Phenolic components of the plant cell wall. Int Rev Cytol 1994;151:229-67.
62. Hellio C, De La B, Dufosse D, Le G, Bourgougnon N. Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. Mar Environ Res 2001;52(3):231-47.
63. Lam C, Harder T. Marine macroalgae effect abundance and community richness of bacterioplankton in close proximity. J Phycol 2007;43:874-81.
64. Maximilien R, De Nys R, Holmstrom C, Gram L, Givskov M, Crass K, *et al.* Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. Aquat Microb Ecol 1998;15(3):233-46.
65. Lanciotti R, Gianotti A, Patrignani A, Belleti N, Guerzoni ME, Gardini F. Use of natural aroma compounds to improve shelf-life of minimally processed fruits. Trends Food Sci Technol 2004;15:201-8.
66. Xu R, Zhao W, Xu J, Shao B, Qin G. Studies on bioactive saponins from Chinese medicinal plants. Adv Exp Med Biol 1996;5:371-82.
67. George F, Zohar K, Harinder PSM, Klaus B. The biological action of saponins in animal systems: a review. Br J Nutr 2002;88(6):587–605.
68. Rao P, Parekh KS. Antibacterial activity of Indian seaweeds. Phykos 1981;23:216-21.