



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India

Solomon Jeeva, Johnson Marimuthu @ Antonisamy*, Cosman Domettila, Babu Anantham, Mony Mahesh

Centre for Biotechnology, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India

ARTICLE INFO

Article history:

Received 15 January 2012

Received in revised form 23 January 2012

Accepted 30 March 2012

Available online 28 April 2012

Keywords:

Phytochemistry

Seaweeds

*Ulva**Sargassum*

ABSTRACT

Objective: To explore the phytochemical constituents of *Ulva reticulata* (*U. reticulata*) and *Sargassum wightii* (*S. wightii*). **Methods:** The preliminary phytochemical screening was performed by Harborne method. **Results:** The results of the phytochemical screening revealed the presence of steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, coumarins, and xantoproteins in the extracts of *U. reticulata* and steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, carboxylic acid, coumarins, and xantoproteins were detected in the extracts of *S. wightii*. **Conclusions:** The solvent extracts of *U. reticulata* and *S. wightii* show a number of metabolites presence, further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

1. Introduction

Seaweeds are primitive on-flowering plants without true root stem and leaves. They include one of the commercially important marine renewable prosperity. Seaweeds have been used as food stuff in the Asia diet for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals, which are essential nutrition for human^[1]. Bio-stimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Seaweeds have recently received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial

activities. Many metabolites isolated from marine algae have bioactive efforts^[2–4]. Among different compounds with functional properties, antioxidants are the most widely studied. Oxidative stress is an important factor in the pathological genesis, from cancer to cardiovascular and degenerative disease. To date, there are quite a lot of reports on antibacterial activity of solvent extracts from marine algae. However, there are very few reports pertaining to antifungal activity of crude solvent extracts from the seaweeds representing Phaeophyceae and Rhodophyceae^[5]. Seaweeds have been considered as potential source of marine medicinals including antimicrobial, cancer therapies^[6] hypocholesterolemic and anthelmintic substances. Many scientists also reported antimicrobial activities of marine algae^[7–12]. Several compounds from the ocean show pharmacologica activities and bioactive compounds, primarily for treating deadly diseases like cancer, acquired immuno deficiency syndrome, arthritis *etc.*, while some compounds have been used to treat inflammation *etc.* Historically seaweeds provide essential economic, environmental, aesthetic, and cultural benefits to humanity^[6]. For centuries, many of the seaweed secondary metabolites (SSM) have been used for traditional medicines due to their therapeutic potentials^[13]. Recent studies have shown that marine algae are tremendous source of

*Corresponding author: Johnson Marimuthu @ Antonisamy, Centre for Biotechnology, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, India.

E-mail: ptcjohnson@gmail.com

structurally novel and diverse array of marine secondary metabolites^[11,12]. Marine algae are continuously exposed to many biotic and abiotic pressures which influence the organism's physiology, and in turn leads to the production of multifunctional natural secondary metabolites. So far, more than 2400 SSM are described and many of the SSM are natural blueprints for the development of new drugs^[14,15]. Several of these compounds are constitutive, existing in biologically active forms in healthy seaweeds. The major secondary metabolites produced by seaweeds are halogenated compounds^[16] displaying antibacterial, antifungal, antiviral, antifouling and anti-feedent properties. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms. Few reports are available on the biopotential and biochemical studies on the seaweeds from Gulf of Mannar and peninsular coast of India^[17]. With this knowledge the present study was aimed to develop standard method for the extraction of *Ulva reticulata* (*U. reticulata*) and *Sargassum wightii* (*S. wightii*) against the selected pathogenic microorganisms.

2. Materials and methods

2.1. Collection of samples

The samples of *U. reticulata* and *S. wightii* were collected by handpicking at Rasthacaud coastal waters (Gulf of Mannar Coast, Lat N 08008'308'' E77032'80''). 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. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out room temperature in shade. Shade dried samples were grounded into fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

Table 1. Phytochemical screening of different solvents extract of *U. reticulata*.

Phytochemical	Acetone	Benzene	Chloroform	Ethanol	Petroleum ether	H ₂ O
Alkaloids	–	–	–	–	–	–
Phenol	+	+++	+	+	++	+
Flavonoids	–	+	+	–	++	+++
Saponins	–	+++	+++	–	++	–
Protein	–	–	–	–	–	–
Quinone	–	–	–	–	–	–
Steroids	++	+	–	+	–	–
Tannin	+	–	+	+	+	+++
Xanthoprotein	++	–	–	–	–	–
Carboxylic acid	–	–	–	–	–	–
Coumarins	–	–	+++	–	+	+
Carbohydrates	–	–	+++	–	–	–

2.2. Preparation of extracts

The powdered samples (2 g) and packed in Soxhlet apparatus and extracted with ethanol, acetone, petroleum ether, chloroform, benzene and water for 8 h. The crude extracts were weighed and deep frozen (–20°C) until tested. The preliminary phytochemical screening was performed by Harborne method^[18].

3. Results

3.1. *U. reticulata*

By preliminary phytochemical screening of twelve different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannin, flavonoids, anthraquinone, carbohydrates, carboxylic acid, coumarins, proteins and xantoproteins) were tested in six different extracts. Thus out of (6×12 = 72) tests for the presence or absence of the above compounds, only 26 gave positive results and the remaining 46 gave negative results. The 26 positive results showed the presence of steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, coumarins, and xantoproteins. Alkaloids, proteins, quinone and carboxylic acid did not show any positive result for their presence in any of the six extracts tested.

Phenolic group, tannins showed the maximum presence in five different extracts followed by flavonoids in 4 extracts, steroids, saponins and coumarins in 3 different extracts. Among the six different extracts, chloroform extract showed the presence of maximum number (6) of compounds. Next to that, petroleum extracts showed 5 compounds. Acetone, water and benzene extracts showed 4 compounds each and ethanol extracts showed only three compounds (Table 1).

3.2. *S. wightii*

The preliminary phytochemical studies on acetone,

Table 2.
Phytochemical screening of different solvents extract of *S. wightii*.

Phytochemical	Acetone	Benzene	Chloroform	Ethanol	Petroleum ether	H ₂ O
Alkaloids	–	–	–	–	–	–
Phenol	++	+++	+	+++	+	++
Flavonoids	–	+	++	+	–	+
Saponins	–	+++	+++	–	+++	–
Protein	–	–	–	–	–	–
Quinone	–	–	–	–	–	–
Steroids	+	+++	+++	–	–	–
Tannin	+	–	–	+	+	++
Xanthoprotein	++	–	–	–	–	–
Carboxylic acid	–	+	–	–	–	–
Coumarins	++	++	+	–	+	–
Carbohydrates	–	–	+++	+	–	+

(–) absent; (+) low; (++) average; (+++) high.

ethanol, petroleum ether, benzene, chloroform and water extracts of *S. wightii* revealed the presence of 31 gave positive results out of 72. The 31 positive results showed the presence of steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, carboxylic acid, coumarins, and xanthoproteins. Alkaloids, proteins and quinone failed to show their presence in any of the six extracts tested (Table 2). Phenolic group showed the maximum presence in six different extracts followed by flavonoids, tannins and coumarins in 4 extracts, steroids, saponins and carboxylic acid also presented in 3 different extracts. Among the six different extracts, chloroform extract showed the presence of maximum number (6) of compounds. Next to that, petroleum extracts showed 5 compounds. Acetone, water and benzene extracts showed 4 compounds each and ethanol extracts showed only three compounds (Table 2).

4. Discussion

The preliminary phytochemical screening clearly tallies with the colour of the extracts which are all predominantly light or dark green or yellowish–green. These green colours are mainly due to the presence of different kinds of chlorophyll pigments (Chlorophyll a & b) along with other common pigments like carotenoids. But the ethanol extract and chloroform extract are little different in colour *i.e.*, the ethanol extract is reddish–green under ordinary light and brownish green in UV light and the chloroform extract is orange–green under ordinary light.

The seaweeds known as medicinal, are rich in secondary metabolites which includes alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry^[17]. The present study observations were directly coincided with the previous observation. 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 have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications^[19]. In the present study we revealed the tannins and xanthoproteins presence in *U. reticulata* and *S. wightii*. Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce 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^[20,21]. Coumarin has been used as anti-coagulant drugs and to treat lymphedema^[22]. Flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals highlights many of the flavonoid health-promoting functions in organism, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity^[23,24]. Manilal *et al*^[25] studied the antimicrobial potential of marine organisms collected from the southwest coast of India against multiresistant human and shrimp pathogens. To supplement the previous observation, in the present study we reported the preliminary phytochemical studies on the *U. reticulata* and *S. wightii*. In the present study we observed the presence of steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, coumarins, and xanthoproteins in the extracts of *U. reticulata* and steroids,

phenolic groups, saponins, tannin, flavonoids, carbohydrates, carboxylic acid, coumarins, and xantoproteins were detected in the extracts of *S. wightii*. In addition to the previous observation, the present study revealed and supplemented the phyto-constituents from the extracts of *U. reticulata* and *S. wightii*. From the results, it can be concluded that the solvent extracts of *U. reticulata* and *S. wightii* showed a number of metabolites presence, further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Mohamed Fayaz KK, Namitha KN, Chidambara Murthy M, Mahadeva Swamy R, Sarada Salma Khanam PV, Subbarao, et al. Chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezii* (Doty). *J Agri Food Chem* 2005; **53**: 792–797.
- [2] Oh KB, Lee JH, Chung SC, Shin J, Shin HJ, Kim HK, et al. Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives. *Bioorganic & Med Chem Lett* 2008; **18**: 104–108.
- [3] Somepalli Venkateswarlu Gopala K, Panchagnula Aditya L, Gottumukkala Gottumukkala V, Subbaraju. Synthesis, structural revision, and biological activities of 4'-chloroaurone, a metabolite of marine brown alga *Spatoglossum variabile*. *Tetrahedron* 2007; **63**(29): 6909–6914.
- [4] Yang RY, Li CY, Lin YC, Peng GT, She ZG, Zhou SN. Lactones from a brown alga endophytic fungus (No. ZZ36) from the South China Sea and their antimicrobial activities. *Bioorg & Med Chem Lett* 2006; **16**(16): 4205–4208.
- [5] Manilal A, Sujith S, Selvin J, Kiran GS, Shakir C, Lipton AP. Antimicrobial potential of marine organisms collected from the southwest coast of India against multiresistant human and shrimp pathogens. *Scientia Marina* 2010; **74**(2): 287–296.
- [6] Thirumaran G, Anantharaman P. Antibacterial activity and antifungal activities of marine macro alga (*Hydroclathrus clathratus*) from the Gulf of Mannar Biosphere Reserve. *Environ & Ecol* 2006a; **24**(1): 55–58.
- [7] Thirumaran GP, Baskar V, Anantharaman P. Antibacterial and antifungal activities of seaweed (*Dictyota dichotoma*) from the Gulf of Mannar Biosphere Reserve. *J Ecotoxicol Environ & Ecol* 2006b; **24**(1): 37–40.
- [8] Salvador N, Gomez-Garreta A, Lavelli L, Ribera L. Antimicrobial activity of Iberian macro algae. *Mar Sci* 2007; **71**: 101–113.
- [9] Manivannan K, Karthikai devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. *Asian Pac J Trop Biomed* 2011; 117–123.
- [10] Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Ann Microbiol* 2008; **58** (3): 535–541.
- [11] Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, Gandhimathi R, et al. Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. *Ann Microbiol* 2009; **59** (2): 207–219.
- [12] Dhargalkar VK, Neelam P. Seaweed: Promising plant of the millennium. *Sci Cul* 2005; **71**: 60–66.
- [13] Fitton JH. Antiviral properties of marine algae. In: Critchley AT, Ohno M, Largo DB. *World seaweed resources*. Workingham, UK: Windows and Macintosh. ETI Information Services; 2006, p. 7.
- [14] El-Baroty GS, Moussa MY, Shallan MA, Ali MA, Sabh AZ, Shalaby EA. Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: *Asparagopsis taxiformis* (Delile) Trevisan. *J Appl Sci Res* 2007; **3**: 1825–1834.
- [15] Al-Fadhli A, Wahidulla S, D'Souza L. Glycolipids from the red alga *Chondria armata* (Kütz.) Okamura. *Glycobiol* 2006; **6**: 902–915.
- [16] Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2007; **24**: 31–86.
- [17] Eluvakkal T, Sivakumar SR, Arunkumar K. Fucoidan in some Indian brown seaweeds found along the coast of Gulf of Mannar. *Int J Botany* 2010; **6**(2): 176–181.
- [18] Harborne JB. *Phytochemical methods*. London: Chapman and Hall; 1998.
- [19] 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): 2056–2071.
- [20] George F, Zohar Kerem, Harinder PSM, Klaus Becker. The biological action of saponins in animal systems: a review. *Brit J Nutr* 2002; **88**(6): 587–605.
- [21] Xu R, Zhao W, Xu J, Shao B, Qin G. Studies on bioactive saponins from Chinese medicinal plants. *Adv Exp Med & Biol* 1996; **404**: 371–382.
- [22] [Online] Available from: <http://en.wikipedia.org/wiki/Coumarin>
- [23] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2005; **26** (5): 343–356.
- [24] De Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, et al. Phosphoprotein levels, MAPK activities and NFkappaB expression are affected by fisetin. *J Enzyme Inhib Med Chem* 2007; **22** (4): 439–444.
- [25] Manilal A, Sujith S, Selvin J, Kiran GS, Shakir C, Lipton AP. Antimicrobial potential of marine organisms collected from the southwest coast of India against multiresistant human and shrimp pathogens. *Scientia Marina* 2010; **74**(2): 287–296.