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Research Paper

PHYTOCHEMICAL ANALYSIS OF MARINE MACROALGA *Caulerpa racemosa* (J. AGARDH) (CHLOROPHYTA - CAULERPALES) FROM TIRUNELVELI DISTRICT, TAMILNADU, INDIA

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Abstract

Phytochemical screening providing important ideas for the development of new drugs against deadly diseases. Recently, a number of studies have been reported on the phytochemistry of marine algae across the world. The marine macroalga *Caulerpa racemosa* methanol solvent extract was prepared by cold percolation method. The shad dried seaweed powder and its crude extract were tested for organoleptic properties, fluorescence analysis, and qualitative phytochemical analysis for the identification of active bio-molecules. Methanol solvent extract of *Caulerpa racemosa* showed the presence of a number of metabolites such as phenolic groups, saponins, tannin, flavonoids, carbohydrates, reducing sugars, Xanthoproteins. This suggest that the *Caulerpa racemosa* seaweed can be used as antimicrobial (antiviral, antifungal and antibacterial), antiparasitic, anti-inflammatory, antifeedent, antioxidant, antiallergenic, antithrombic and antiulcer agents in the near future.

Key words: Seaweed, Phytochemistry and Secondary metabolites.

INTRODUCTION

Seaweeds are wonder plants of the sea and considered as medicinal food of the 21st century. Marine macroalgae, popularly known as seaweeds are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and are grouped into green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae. Marine algae from Indian coasts amounting to 844 species (including forma and varieties) are distributed among 217 genera. Seaweeds are primitive non-flowering plants without true root, stem and leaves. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries, backwaters and lagoons on solid substrates such as rocks, dead corals, pebbles, shells, mangroves and other plant materials (Anatharaman, *et al.,* 2007; Sakthivel, 2007).

Marine organisms are source material for structurally unique natural products with pharmacological and biological activities (Faulkner, 2001). Among marine organisms, the macroalgae occupy an important place as a source of biomedical compounds (Selvin and Lipton, 2004; Manilal *et al*., 2009 [ab] and Manilal *et al*., 2010). About 2400 natural products have been

isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae (Faulkner, 2001).

Seaweeds known as medicinal are rich in secondary metabolites which include 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 (Eluvakkal *et al*., 2010). Recently, their value as a source of novel bioactive substances has grown rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Kim and Wijesekara, 2010; Wijesekara and Kim, 2010; Wijesekara *et al.,* 2010 and Wijesekara *et al.,* 2011).

In recent years, the seaweeds serve as an important source of bioactive natural substances (Plaza, *et al.,* 2008 and Smit, *et al.,* 2004). Moreover, many metabolites, which isolated from marine algae, have shown to possess bioactive effects (Faulkner, 2002 and Kim *et al*., 2005). Therefore, recently a new trend has been arisen to isolate novel bioactive compounds and constituents from edible seaweeds. (Li, *et al*., 2011). Phytochemical analysis of seaweeds can help the manufacturers for identification and selection of raw materials for drug production.

Seaweeds are the economic potential resource in marine environment and it will be a great significance in drug development (Fuesetani, 2000). Seaweeds known as medicinal are rich in secondary metabolites which include 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 (Eluvakkal *et al*., 2010).

A greater diversity in biochemical composition of seaweeds paves way to explore variety of compounds in their bodily composition with a wide range of physiological and biochemical characteristics, many of which are rare or absent in other taxonomic groups (Holdt and Kraan, 2011). The secondary metabolites of seaweeds such as Isoprenoids (terpenes, carotenoids, steroids), polyketides, phlorotannins, amino-acid derived natural products (alkaloids), and shikimates (flavonoids) have always attracted the interest of biochemists because of their diversity as compared with those present in the leaves of higher plants (Manilal *et al*. (2009).

Seaweeds were rich in dietary fiber (>50% dry weight), particularly in the soluble form (Mabeau *et al*., 1993); Fleury *et al*. (1991) reported that the physico-chemical properties of seaweed powder could be assumed to reflect those of the present fibre. Moreover, since seaweed proteins are closely related to the cell wall polysaccharides (Fleurence, 1999), they may also play a role in the physicochemical properties, such as water holding.

Phytochemical screening providing important ideas for the development of new drugs against deadly diseases. Recently, a number of studies have been reported on the phytochemistry of plants across the world (Edeoga, *et al*.,2005; Aliyu, *et al*., 2008; Ayoola, *et al*.,2008; Johnson, *et al*., 2008; Maridass, *et al*., 2008a; Maridass, *et al.,* 2008b; Ujowundu, *et al*., 2010; Rafia Rasool, *et al*., 2010; Usha and Bopaiah, 2011 and Hamad, *et al.,* 2011), in particular seaweeds (Poonam, 2011; Ganga Rao *et al.,* 2011; Jayasree *et al.,* 2012; Venkatesh *et al.,* 2011; Johnson, *et al* 2012, and Rajasulochana, *et al*., 2009) were reported.

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. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Its potent water soluble antioxidants and free radical scavengers prevent oxidative cell damage and have strong anti-cancer activity. They show anti-allergic, anti-inflammatory, anti- microbial and anti-cancer activity (Cushnie and Lamb 2005; De Sousa *et al.,* 2007 and Yadav *et al*., 2011). Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammtory properties (Arts *et al.,* 2005 and Scalbert *et al*., 2005).

The secondary metabolites of seaweeds have always attracted the interest of biochemists because of their diversity as compared with those present in the leaves of higher plants. Isoprenoids (terpenes, carotenoids, steroids), polyketides (Phlorotannins), amino-acid-derived natural products (alkaloids), and shikimates (flavonoids) are the major groups of

secondary metabolites found in algae (Mendis and Kim, 2011). In this background, the present study intended to evaluate the qualitative and phytochemical (secondary metabolites) analysis in the seaweed *Caulerpa racemosa* collected from the Tirunelveli District Tamil Nadu, India was studied.

MATERIALS AND METHODS

Marine Alga *Caulerpa racemosa* (J. Agardh)

Caulerpa racemosa Thalli are pale green 5-6 cm tall. Plants in larger or smaller group possess rhizome with creeping, cylindrical and highly branched. Rhizoids arise from the lower parts of prostrate rhizome. Crowded with bud-like branchlets in spherical shape, sometimes oval not constricted at the base. They are found growing on sandy, rocky or silt-covered substrate attached by numerous rhizoids arising from the creeping stolon. It was observed that the thalli were rarely associated with other macroalgae (Iyengar, (1933) and Umamaheswaran Rao, 1987[ab]). *Caulerpa racemosa* distributed in Maharashtra (Bombay, Malwan), Gujarat (Dwarka, Okha, Sauraqshtra, Veraval), Tamil Nadu (Tirunelveli, Idinthkarai; Kuttapuli, Palk Bay, Pamban, Mandapam, Tuticorin, Krusadai Island,(Gulf of Mannar), Kerala(Kovalam), Andhara Pradesh(Visakapatnam), Goa, Karnataka, Lakshadweep Island and Andaman and Nicobar Islands.

Collection of *Caulerpa racemosa* (J. Agardh)

The macro alga *Caulerpa racemosa* were collected from, Kuttapuli Tirunelveli District, Tamil Nadu, immediately after collection, the macro alga washed in sea water and the epiphytes, associated organisms and other extraneous matter were removed. After subsequent washing in fresh water the alga were shade dried for two weeks continuously. The shade-dried seaweed was partially powdered using domestic blender and stored in air tight container for further experiments.

Preparation of Extracts

From these stock, secondary metabolites of seaweed (100g), was extracted successively using (150mL) solvent methanol the sample were kept in dark for 96 hour. After incubation, the extract thus obtained was decanted and filtered. The clear extract was subsequently concentrated using rotary vacuum evaporator and kept in dark bottles in 4^{o} C until use (Johnson *et al.,* 2012.)

Physico-Chemical parameters of seaweed

The *C. racemosa* powder was used for physico-chemical, fluorescent and phyto-chemical analyses. The procedures recommended in Indian Pharmacopoeia (Anonymous, 1966; 1985; 1996) were followed.

Fluorescence Analysis

Fluorescence characteristics of the seaweed powder as such and after treating them with chemical reagents were observed in day light. Fluorescent analyses of the seaweed powder were carried out according to the methods of Chase and Pratt (1949) and Kokoshi *et al.,* (1958) Kokate (2005); Nazish *et al*.(2009); Arun Kumar *et al* .(2011).

Behavior of seaweed powder was treated with different reagents (NaOH, conc. HNO_3, con. HCl, con. H_2SO_4, 5% Acetic acid, 5% $FeCl_3$, ammonia, picric acid) and colour change was observed in natural light (Kay, 1938; Johansen ,1940; Pratt and Chase, 1949 and Nazish *et al*., 2011).

Organoleptic properties

The organoleptic characteristics of alga powered samples namely their appearance and colour in day light, smell and their taste were also studied. Organoleptic evaluation seaweed powder were carried out according to the methods of Kokate *et al*., 2005; Arun Kumar and Paridhavi, 2011).

Qualitative phytochemical Analysis

The different qualitative chemical tests were performed for establishing profile of algal extract for its chemical composition. Qualitative phytochemical analyses were done using the procedures of Kokate *et al.,* (1995) Sofowara (1993), Trease and Evans (1989),Harborne (1973), Brindha,(1991), Edeoga *et al.,* (2005), and Savithramma *et al.,* (2011) .

Swelling capacity (SWC) of seaweed *C. racemosa* was analyzed by the bed volume technique after equilibrating in excess solvent (Kuniak and Marchessault, 1972).Water holding capacity (WHC) of seaweed *C. racemosa* was measured by the modified centrifugation method described by Suzuki *et al*., (1996). The WHC of seaweed was expressed as the weight of grams of water held by 1 g of sample (dry weight).

RESULTS AND DISCUSSION

The Organoleptic characteristics of alga powered sample results revealed that the colour is green, aromatic smell, Tasteless and soft nature when fresh (Table.1**)** The characteristic fluorescent properties or colours emitted by the powdered alga *C. racemosa* before and after treating with various reagents were recorded. The powdered alga as such appeared green under daylight and Ordinary light.

After treating with various reagents, under daylight, it showed different shades of green and brown. However, ordinary light, acidic and alkaline solutions of concentrated HNO_3, HCL , H_2SO_4, 50% H_2SO_4 , 1N HCL , 50% HNO_3 , 5% KOH, MeOH, 1N NaOH, 5% acetic acid, 5% $FeCl_3$, Ammonia and Picric acid, with light green, intense green and dark green colours respectively (Table 2& 3**).** The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the alga *C. racemosa* in its crude form. The ash value indicates the presence of inorganic ions. During ash preparation organic matter gets oxidized and certain amount of volatile elements are lost. (Trease, 1961).

Preliminary phytochemical screening of twenty four different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannins, flavonoids, anthraquinones, reducing sugars, triterpenoids, terpenoids, cardiac glycosides, glycosides, phlobatannins, quinones, aromatic acids, essential oils, anthocyanins, leucoanthocyanins, Emodins, gum and mucilage, carbohydrates, Coumarins, aminoacids and xanthoprotein) were tested in methanolic extract. The results showed that the presence of steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids carbohydrates, reducing sugars, anthraquinones, Emodins coumarins, and xanthoproteins. Alkaloids, triterpenoids, glycosides, cardiac glycosides, aminoacids, quinnone, gum and mucilage, anthocyanins, leucoanthocyanins ,aromatic acids, did not show any positive result for their presence in any of the extract tested. Methanol extract showed the presence of maximum number (10) of compounds. (Table 4).

Table . 1. Organoleptic properties of the alga *C. racemosa*

S. No	Character	When fresh	After drying 20 days	Powder
1	Colour	Green	Light green	Light greenish brown
2	Odor	Aromatic	Aromatic	Aromatic
3	Taste	Tasteless	Tasteless	Sour
4	Texture	Soft	Crispy	Soft

Table 2. Fluorescence analysis of *C. racemosa* powder in different chemical reagents in ordinary light

S. No	**Particulars of treatment**	**Under ordinary light**
1	Powder as such	Green
2	Powder + 50% H_2SO_4	Dark Intense green
3	Powder + 1N HCL	Grey green
4	Powder + 50% HNO_3	Light grey
5	Powder + 5% KOH	Light green
6	Powder +MeOH	Light green
7	Powder + 1N NaOH	Light green

Table. 3. Fluorescence analysis of *C.racemosa* powder in different chemical reagents in natural light

S. No	Drug treatment	Seaweed Powder
1	Powder as such	Green
2	Powder + 1N NaOH	Grey colour
3	Powder + con. HNO_3	Yellowish colour
4	Powder + con. HCL	Intense light green
5	Powder + con. H_2 SO4	Intense black colour
6	Powder + 5% acetic acid	Light green
7	Powder + 5% $FeCl_3$	Light brown
8	Powder + Ammonia	Light green
9	Powder + Picric acid	Yellowish green

Table 4. Qualitative determination of Phytochemicals of alga *C.racemosa*

S. No	Phytochemicals	Inference
1	Alkaloids	–
2	Steroids	–
3	Reducing sugar	++
4	Tannins	++
5	Pholobatanins	+
6	Saponins	+
7	Coumerins	+
8	Flavonoids	+++
9	Terpenoids	–
10	Triterpenoids	–
11	Cardiac Glycosides	–
12	Glycosides	–
13	Anthraquinones	—
14	Phenolic compounds	++
15	Quinones	–

16	Aminoacids	_
17	Essential oil	_
18	Aromatic acid	_
19	Xanthoprotein	**+**
20	Carbohydrates	**+**
21	Anthocyanins	_
22	Leucoanthocyanins	_
23	Emodins	_
24	Gum and mucilage	_

(+++ very good);(++ good);(+) Positive; (-) Negative

In the present study, presence of steroids, phenolic groups, saponins, tannin, flavonoids, carbohydrates, coumarins, and Xanthoproteins. The present study was qualitative analysis of phytochemicals of crude extract was directly coincided with (Rajasulochana, *et al*., 2009; Hebsibah *et al.,* 2011 ; Ganga rao *et al*., 2011; Venkatesh, *et al.,* 2011; Poonam, 2011; Jeeva *et al*.,2012; Jayasree *et al.,* 2012 and Johnson, *et al.,* 2012) reports. These observations cited on phytochemical compounds support our findings on the usefulness of seaweeds in traditional medicament and added a note on the phytochemical history.

Tannins are known to possess general antimicrobial and antioxidant activities (Rievere *et al*., 2009). The present study confirms the presence of tannin in methanol extract of *C.racemosa.* It suggests that the methanol extract of *C. racemosa* with tannins, can be used as antimicrobial agents. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications (Kolodziej and Kiderlen, 2005).

Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory and weight loss, *etc* (Jeeva *et al*., 2012). Saponins have been implicated as bioactive antibacterial agents of plants (Mandal ,*et al*., 2005 and Manjunatha, 2006). Some of these biological properties include antimicrobial, anti-inflammatory, antifungal, antifeedent, and hemolytic effects (George *et al*.,2002; Xu *et al.,* 1996; De-Lucca *et al*.,2005 and Mohanta *et al*., 2007).

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just,1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo, 2000 and Okwu, 2004). The present study confirms the presence of Saponin in methanol extract of *C. racemosa.* It suggests that the methanol extract of *C. racemosa* with saponins , can be used as antimicrobial, anti-inflammatory and antifungal agents.

The present study confirms the presence of steroid in extract of *C. racemosa.* Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu,

2001). It suggests that the extract of *C. racemosa*, can be used as antimicrobial agents. Coumarin has been used as anti-coagulant drugs and to treat lymphedema.

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro.* Flavonoids, the major group of phenolic compounds reported for their antimicrobial and antiviral activity. The presence of Flavonoids in the methanol extract of *C. racemosa.* It suggests that the methanol extract of *C. racemosa* with flavonoids, can be used as antioxidant, antimicrobial, anti-inflammatory, antifungal and anti-cancer agents in the pharmaceutical industry. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Its potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity. They show anti-allergic, anti-inflammatory, anti- microbial and anti-cancer activity (Cushnie and Lamb 2005 ; De Sousa *et al.,* 2007 and Yadav *et al* .,2011).

Seaweed extracts contain rich source of phenolic compounds (Athukorala *et al*., 2003; Heo *et al*., 2005). In the present study showed the presence of phenolics was confirmed by the qualitative analysis in the crude extract of the seaweed. *C .racemosa.* Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties (Duan *et al.,* 2006; Kuda *et al.,* 2007; Wang *et al.,* 2009; Athukorala *et al*., 2006), therefore the seaweed extracts could have potential applications in food industries (Yan *et al*., 1996). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007 and Yadav and Agarwala, 2011).

Capacity increase in the water holding of the *C. racemosa* alga powder with temperature. Such increase was probably related to the increase in the solubility of fibres and proteins (Fleury *et al*., 1991). Also, Wong *et al*. (2000) found that the WHC for the *U. lactuca* seaweed at 37° C lower (9.71 g/g DW) with the result found at 37°C, but also comparable to that of some agricultural by-products (dietary fiber concentrates) (6.30–13.2 g/g DW) reported previously (Grigelmo-Miguel and Martin-Belloso, 1999). Furthermore, the Water Holding Capacity of the *C. racemosa* seaweed samples were also comparable to the Water Holding Capacity of some commercial dietary fiber rich supplements (Goñi and Martin-Carrón, 1998).

Seaweeds were rich in dietary fiber (>50% dry weight), particularly in the soluble form (Darcy-Vrillon, 1993; Mabeau *et al*., 1993); Fleury *et al*. (1991) reported that the physico-chemical properties of seaweed powder could be assumed to reflect those of the present fibre. Moreover, since seaweed proteins are closely related to the cell wall polysaccharides (Fleurence, 1999), they may also play a role in the physicochemical properties, such as water holding.

Water exists in fiber in three forms: it is bound to the hydrophilic polysaccharides; it is held within the fiber matrix or it is trapped within the cell wall lumen. WHC, determined by the centrifugation method used in this study, represented all three types of water associated with the fiber (Fleury *et al*., 1991). Apart from the different water holding ability in fiber, the differences in WHC and SWC among the seaweed samples might be attributed to the different protein conformations and the variations in the number and nature of the water binding sites on the protein molecules. In addition to chemical compositions, some physical properties, such as structure, particle size, porosity, pH, temperature, ionic strength, types of ions in solutions and density were important to the understanding of the different behaviors' of samples during hydration (Fleury *et al.,* 1991).

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and the alga *C. racemosa* are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION

The present study conclude that methanol solvent extract of *C. racemosa* shows a number of metabolites presence, of steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids carbohydrates, reducing sugars, anthraquinones, Emodins coumarins, and xanthoproteins suggest that *C. racemosa* seaweed can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, antioxidant, antiallergenic, antithrombic, anticarcinogenic and anti-ulcer agents in the near future .

CONFLICT OF INTERESTS

The authors do not have any conflict of interests.

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