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PRELIMINARY PHYSICO-CHEMICAL PROPERTIES OF MARINE MACROALGA *SARGASSUM TENERRIMUM* (J. AGARDH) (FUCALES, SARGASSACEAE)

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ABSTRACT:

Sargassum, one of the marine macro algae belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family Sargassaceae and order Fucales. Physico-chemical screening providing important ideas for the development of new drugs against deadly diseases. The marine macroalga *Sargassum tenerrimum* methanol extract was prepared by cold percolation method. The shad dried seaweed powder and its crude extract were tested for swelling capacity, water holding capacity, Organoleptic properties, fluorescence analysis, and qualitative phytochemical analysis for the identification of active bio-molecules. Methanol extract of *Sargassum tenerrimum* showed the presence of a number of metabolites such as steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids carbohydrates, reducing sugars, and Xanthoproteins. This suggest that the *Sargassum tenerrimum* seaweed could be used as antimicrobial (antiviral, antifungal and antibacterial), antiparasitic, anti-inflammatory, antifeedent, antioxidant, antiallergenic, antithrombic and antiulcer agents in the near future.

KEYWORDS: *Seaweed, Phytochemistry and Secondary metabolites.*

INTRODUCTION:

Seaweeds or benthic marine algae are the group of plants that live either in marine or brackish water environment. Seaweeds one of the important marine living resources could be termed as the futuristically promising plants. These plants have been a source of food, feed, agricultural importance and medicines since ancient times. Man has used the sea for many years as a productive source for several economically useful materials, especially to supplement his diet (Prescott 1984)

Sargassum tenerrimum are yellowish in colour, Pyramidal in shape with basal adhesive disc or holdfast from which arises a short primary axis bearing several branches together. The axis is rounded and glabrous, secondary branch fork repeatedly in their turn. Leaves are wide and tapering at the end. They are thin, translucent and linear lanceolate, with toothed margin the midrib is indistinct. Cryptostomata are freely Scattereted on the leaves and receptacles while they are rare on the axis .The receptacles are freely-branched structures.

The brown seaweed*, Sargassum tenerrimum* (J. Agardh) is predominantly distributed in coasts of many Asian countries. *Sargassum* (Phaeophyceae) is a brown seaweed found along the coasts of Japan, China, Pakistan and India (Maharashtra (Bombay), Gujarat (Okha, Adatra reef, Cannanore, Dwarka, Saurashtra, Jalleshwar, Varaval, Gulf of Kutch), Tamil nadu (Tuticorin, Tirunelveli, Cape Comorin, Krusadai Island), Andhra Pradesh (Waltair, Visakhapatnam), Karnataka, Kerala (Travancore). Andaman Island, East Coast, West Coast, Goa, Indian Coast.).

Marine organisms are rich sources of structurally diverse bioactive compounds with various biological activities and their importance as a source of novel bioactive substances is growing rapidly. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds (Aneiros and Garateix, 2004; Barrow and Shahidi, 2008).

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.,* 2010and Wijesekara *et al.,* 2011). The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation (Elena *et al*., 2001; Kim *et al*., 1997; Okai *et al*., 1997; Premila *et al*., 1996) apart from their

potential ecological and industrial significances such as controlling reproduction, settlement and biofouling and feeding deterrents (Selvin, 2002 and Selvin and Lipton 2004).

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 (e.g., terpenes, carotenoids, steroids), polyketides (e.g., phlorotannins), amino-acid-derived natural products (e.g., alkaloids), and shikimates (e.g., flavonoids) are the major groups of secondary metabolites found in algae (Mendis and Kim, 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).

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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; Majaw and Moirangthem, 2009; Mensah, *et al*., 2009; Venkata Ratnam and Venkata Raju, 2009; Chitravadivu, *et al.,* 2009; Benjamin and Christopher, 2009; Maridass, 2010; Okhale, *et al*., 2010; Naga Deepthi, *et al*., 2010; Sunita Dalal, *et al*.,2010; Devmurari and Jivani , 2010; 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.

Phytochemical analysis of seaweed can help the manufacturers for identification and selection of raw materials for drug production. In this background, the present study intended to evaluate the qualitative phytochemical (secondary metabolites) analysis in the seaweed *Sargassum tenerrimum* collected from the Tirunelveli district, Tamil Nadu, India.

MATERIAL AND METHODS :

Collection and Identification

Sargassum tenerrimum (J.Agardh) was collected from Kuttapuli, Koothenkuli, Idinthakarai, Tirunelveli District, Tamil Nadu, India, 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. The alga was identified by Dr P. Paramasivam, HOD, Dept of Botany, Pachaiyappa's College for Men, Kanchipuram.

Preparation of Extract

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 *S. tenerrimum* 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 (1994) and Kokate *et al.,* (1995) Sofowara (1993), Trease and Evans (1989),Harborne (1973), Paris, 1969; Brindha,1991, Edeoga *et al.,* 2005, and Savithramma *et al.,* 2011 .

Physico-chemical properties

Swelling capacity (SWC)

Swelling capacity of seaweed *S. tenerrimum* was analyzed by the bed volume technique after equilibrating in excess solvent (Kuniak and Marchessault, 1972). To 200 mg of seaweed sample in a 50 ml measuring cylinder, 20 ml of de- ionized water were added and the mixtures were then vigorously stirred. The measuring cylinder was left to stand for 24 h at 25 and 37^{o}C. Swelling volume was measured and expressed as milliliters of swollen sample per g of sample (DW).

Water-holding capacity (WHC)

Water holding capacity of seaweed *S. tenerrimum* was measured by the modified centrifugation method described by Suzuki *et al*., (1996). Twenty mL of de-ionized water were added to each centrifuged tube containing 200 mg of seaweed sample. Then the tubes were shaken in a shaking culture bath for 24 h at 25 and 37^{o}C. After centrifuging at 14,00RPM for 30 min, the supernatant was discarded and the moisture content of pellet was determined after dehydration in an oven for 2 h at 120^{o}C. The WHC of seaweed was expressed as the weight of grams of water held by 1 g of sample (dry weight).

RESULT AND DISCUSSION:

The characteristic fluorescent properties or colours emitted by the powdered alga *S. tenerrimum* 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 Na OH, 5% acetic acid, 5% $FeCl_3$, Ammonia and Picric acid, with light green, intense green and dark green colours respectively (Table 1& 2**).** The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the alga *S. tenerrimum* in its crude form. The

Organoleptic characteristics of alga powered sample results revealed that the colour is brown, aromatic smell, Sour taste and soft nature when fresh (Table.3**)**

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 crude extract. Thus out of (1×24 =24) tests for the presence or absence of the above compounds (Table 4).

The results showed that the presence of steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids carbohydrates, reducing sugars, 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 extracts tested. Steroids, Flavonoids, Phenolic groups, and tannins showed the maximum presence in the extract (Table 4).

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 *S. tenerrimum* 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).

Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammtory properties (Arts *et al.,* 2005 and Scalbert *et al*., 2005). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger 2001).

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).

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Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. Saponins are known to produce inhibitory effect on inflammation. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. 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).

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). Coumarin has been used as an anti-coagulant drug and to treat lymphedema.

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).

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. It's have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens.

Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. Its ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals highlights many of the flavonoids health-promoting

functions in organism, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA (Cushnie and Lamb 2005). They also are effective antioxidant and show strong anticancer activities (Ali *et al*., 2008; Marjorie, 1996; Salah *et al*., 1995; Del-Rio *et al*.,1997 and Okwu, 2004).

The presence of Flavonoids in the methanol extract of *S. tenerrimum* . It suggests that the methanol extract of *S. tenerrimum* 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).

The present study, reveals the water holding capacity of seaweed *Sargassum tenerrimum* 13.5g and 15.5g of water per gram of dry matter at 25 ^{o}C and 37^{o} C, respectively. The swelling capacity of the *Sargassum tenerrimum* seaweed was about 1.0 mL/ g and 1.5 mL/g at 25^{o}C and 37^{o}C respectively. 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).

Capacity increase in the water holding of the *S. tenerrimum* 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^{o} C lower (9.71 g/g DW) with the result found at 37^{o}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 *S. tenerrimum* 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.The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and the alga *S. tenerrimum* are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION:

From the results, it can be concluded that the Water holding capacity (WHC), Swelling Capacity (SW) of the marine macro alga *Sargassum tenerrimum* was higher than compare to other seaweeds. Organoleptic properties of the seaweed *Sargassum tenerrimum* results revealed that brown colour, sour taste, aromatic smell and soft in nature and methanol extract of *Sargassum tenerrimum* showed a number of metabolites presence, of steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids carbohydrates, reducing sugars, and xanthoproteins suggest that *S. tenerrimum* seaweed would be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcinogenic and anti-ulcer agents in the near future.

CONFLICT OF INTERESTS

The authors do not have any conflict of interests.

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Table .1. Fluorescence analysis of *Sargassum tenerrimum* powder in different chemical reagents in natural light

S. No	Drug treatment	*S. tenerrimum* Powder
1	Powder as such	Brown colour
2	Powder + 1N NaOH	Black colour
3	Powder + con. HNO_3	Brownish Yellow colour
4	Powder + con. HCL	Intense light green colour
5	Powder + con. H_2SO4	Intense black colour
6	Powder + 5% acetic acid	Light Brown colour
7	Powder + 5% $FeCl_3$	Light brown colour
8	Powder + Ammonia	Light green colour
9	Powder + Picric acid	Dark Yellow colour

Table 2. Fluorescence analysis of *Sargassum tenerrimum* powder in different chemical reagents in ordinary light

S. No	Particulars of treatment	Under ordinary light
1	Powder as such	Brown colour
2	Powder + 50% H_2SO_4	Dark green colour
3	Powder + 1N HCL	Blackish green colour
4	Powder + 50% HNO_3	Golden Yellow colour
5	Powder + 5% KOH	Reddish brown colour
6	Powder +MeOH	stand stone colour
7	Powder + 1N NaOH	Light green colour

Table.3. Organoleptic properties of the alga *S. tenerrimum*

S. No	Character	When fresh	After drying 20 days	Powder
1	Colour	Brownish yellow	Light yellow	Light brownish yellow
2	Odor	Aromatic	Aromatic	Aromatic
3	Taste	Tasteless	Sour	Sour
4	Texture	Soft	Crispy	Soft

Table 4. Qualitative determination of phytochemical in methanolic extract of *Sargassum tenerrimum.*

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	_

(+) Positive; (-) Negative