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CHEMICAL PROFILE OF SELECTED GREEN SEAWEEDS OF SOUTHERN COAST OF TAMIL NADU, INDIA

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ABSTRACT

The present study was aimed to determine the phytochemical constituents of the following seven selected green seaweeds viz., *Caulerpa corynephora* Montagne, *Caulerpa scalpelliformis* (R. Br.) Weber-van Bosse, *Chaetomorpha antennia* (Bory de Saint-Vincent) Kutzing, *Enteromorpha compressa* (L.) Grev., *Halimeda macroloba* Decsne., *Ulva fasciata* Delile and *Ulva lactuca* Linn. To reveal the phytoconstituents composition of selected seven green seaweeds, phytochemical analysis was performed. Thus out of 350 (7 x 5 x 10 = 350) tests, 95 positive results show the presence of steroids (21), alkaloids (8), phenolics (8), flavanoids (5), saponins (4), tannins (29), cardiac glycosides (15) and triterpenoids (5). Amino acids and anthraquinone did not show any positive result for their presence in any of the tested five extracts of seaweeds. Among the five different extracts tested the ethanolic extracts showed maximum presence of metabolites. The fluorescent characteristics of the *C.corynephora, C. scalpelliformis, C. antennia, E. compressa, H. macroloba, U. fasciata* and *U. lactuca* were observed under UV and fluorescence light.

KEYWORDS: Green seaweeds; Phytocemistry; Chemical profile.

INTRODUCTION

For centuries, seaweed has been of botanical, industrial and pharmaceutical interest. In recent years research on the chemistry of seaweeds has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties (Troell *et al*., 2006; Leary *et al*., 2009; Hela *et al*., 2011; Prabha *et al*., 2013; Papenfus *et al*., 2013). Seaweeds offer a wide range of therapeutic possibilities both internally and externally. Seaweeds have extensive profile source of secondary metabolites. Although a majority of these) are terpenes (60%), but some fatty acids are also common (20%) with nitrogenous compounds. Since then, numerous studies have been carried out to detect and extract antimicrobial compounds from marine algae of all three groups viz. Rhodophyceae, Phaeophyceae and Chlorophyceae (Biard *et al*., 1980). Seaweeds have been considered as potential source of marine medicinal including antimicrobial, anticancer (Dhargalkar and Neelam, 2005) hypocholesterolemic and anithelminthic substances. Many scientists also reported antimicrobial activities in marine algae (Thirumaran *et al*., 2006 and 2006a; Salvador *et al*., 2007; Shanmughapriya *et al*., 2008; Manilal *et al*., 2009; Manivannan *et al*., 2011). Several compounds from the ocean show pharmacological activities and bioactive compounds, primarily for treating deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS) and arthritis etc., while some compounds have been used to treat inflammation. Historically seaweeds provide essential economic, environmental, aesthetic and cultural benefits to humanity (Dhargalkar and Neelam, 2005).

The ancient literatures indicate that seaweed is still employed in folk medicine in many parts of the world as treatments of a variety of diseases. The Japanese and Chinese have used seaweeds to treat goiter and other glandular problems since 300 BC. The Romans used them in the treatment of wounds, burns and rashes. For centuries, many of the seaweed secondary metabolites (SSM) have been used for traditional medicines due to their therapeutic potentials (Fitton, 2006). Recent studies have shown that marine algae are tremendous source of structurally novel and diverse array of marine secondary metabolites (Shanmughapriya *et al*., 2008; Manilal *et al*., 2009). A number of biologically active compounds with varying degrees of action, such as antitumour, anticancer, cytotoxic (Mayer, 1999; Mayer and Lehmann, 2001; Mayer and Gustafson, 2003; 2004; 2006 and 2008). antimicrotubule, antiproliferative photoprotective, as well as antibiotic and antifouling properties have so far been isolated from marine sources (Villa *et al*., 2010; Mayer *et al*., 2007; Blunt *et al*., 2011).

Some of these bioactive secondary metabolites of marine origin with strong antibacterial, antifungal and antiviral activities, are currently in intense use as antibiotics and may be effective against infectious diseases such as human immunodeficiency virus (HIV) and conditions of multiple bacterial infections (penicillin, cephalosporins, streptomycin and vancomycin). Marine organisms are under persistent threat of infection by resident pathogenic microbes including bacteria, and in response they have engineered complex organic compounds with antibacterial activity from a diverse set of biological precursors. The diluting effect of the ocean drives the construction of potent molecules that are stable to harsh salty conditions. Members of each class of metabolites, such as ribosomal and nonribosomal peptides, alkaloids, polyketides and terpenes, have been shown to exhibit antibacterial (Nair *et al*., 2007; Mayer *et al.*, 2009); and antiviral activity (Hughes *et al*., 2010; Yasuhara, 2010; Smith *et al.*, 2010). With this knowledge the present study was aimed to determine the phytochemical constituents of the following seven selected green seaweeds viz., *Caulerpa corynephora* Montagne, *Caulerpa scalpelliformis* (R. Br.) Weber-van Bosse, *Chaetomorpha antennia* (Bory de Saint-Vincent) Kutzing, *Enteromorpha compressa* (L.) Grev., *Halimeda macroloba* Decsne., *Ulva fasciata* Delile and *Ulva lactuca* Linn.

MATERIALS AND METHODS

For the phytochemcial, the seaweeds *Caulerpa corynephora* Montagne, *Caulerpa scalpelliformis* (R. Br.) Weber-van Bosse, *Chaetomorpha antennia* (Bory de Saint-Vincent) Kutzing, *Enteromorpha compressa* (L.) Grev., *Halimeda macroloba* Decsne., *Ulva fasciata* Delile and *Ulva lactuca* Linn. were collected by handpicking. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected samples were then thoroughly washed with tap water followed by distilled water. The washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade to remove the excess water contents. The shade dried samples were ground to fine powder using mechanical grinder. The powdered samples were stored for further use.

The dried and powered seaweed materials (30 g) were extracted successively with 180 ml of hexane, petroleum ether, chloroform and ethanol by using Soxhlet extractor for 8 hrs at a temperature not exceeding the boiling point of the solvent. The aqueous extract was prepared by directly boiling the powder with distilled water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -

20º C until further tests. The different extracts were tested for steroids, amino acids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, anthraquinones, terpenoids and cardiac glycosides. Phytochemical screening of the extracts was carried out according to the standard methods (Harborne, 1998).

RESULTS

Phytochemical analysis of *Caulerpa corynephora*

Among the five different solvents used to extract the phytochemicals from *Caulerpa corynephora*, more number of metabolites was observed in ethanolic extract (Table 1). Tannins were observed in all the five extracts of *C. corynephora*. Steroids and cardiac glycosides showed their presence in hexane, petroleum ether, chloroform and ethanol but not in aqueous extract. Flavonoids were observed in hexane and petroleum ether extracts. Phenolics, terpenoids and alkaloids were present only in ethanolic extract.

Phytochemical studies of *Caulerpa scalpelliformis*

Among the five solvents used to extract the phytochemicals from *C.scalpelliformis*, more number of metabolites was observed in ethanolic extract of *C.scalpelliformis* (Table 1). Steroids, alkaloids, phenolics, tannins, cardiac glycosides and terpenoides were observed in the ethanolic extract of *C. scalpelliformis.* Tannins were observed in all the extracts. Steroids and cardiac glycosides showed their presence in hexane, petroleum ether, chloroform and ethanolic extracts but not in aqueous extract. Phenolics were observed in chloroform and ethanolic extracts but not observed in other extracts.

Phytochemical analysis on *Chaetomorpha antennia*

Among the five solvents used to extract the phytochemicals from *Chaetomorpha antennia*, more number of metabolites were observed in the ethanolic extract of *C. antennia* (Table 1). Steroids, alkaloids, phenolics, saponins and tannins were observed in the ethanolic extract of *C. antennia.* However steroids were observed in the petroleum ether, chloroform, ethanolic and aqueous extract but not in hexane extract.

Phytochemical studies on *Enteromorpha compressa*

Among the five solvents used to extract the phytochemicals from *E.compressa*, more number of metabolites was observed in ethanolic extract (Table 1). Steroids, alkaloids, phenolics, terpenoids and tannins were observed in the ethanolic extract of *E. compressa.* However tannins were observed in all the five extracts. Steroids were observed in petroleum ether, chloroform and ethanolic extracts but not in hexane and aqueous extracts. Alkaloids were observed in petroleum ether and ethanolic extracts.

Phytochemical analysis on *Halimeda macroloba*

Among the five solvents used to extract the phytochemicals from *H. macroloba*, more number of metabolites was observed in ethanolic extract (Table 1). Steroids, alkaloids, phenolics, flavonoids, saponins, tannins and cardiac glycosides were observed in the ethanolic extract of *H. macroloba*. However tannins were observed in all the five extracts used. Steroids, tannins and cardiac glycosides showed their presence in chloroform and ethanolic extracts and failed to observe in hexane, petroleum ether and aqueous extracts.

Phytochemical studies on *Ulva fasciata*

Among the five solvents used to extract the phytochemicals from *U. fasciata*, more number of metabolites was observed in ethanolic extract (Table 1). Steroids, alkaloids, phenolics, terpenoids, saponins and cardiac glycosides were observed in the ethanolic extract of *U. fasciata*. However tannins were observed in the hexane, petroleum ether, chloroform and aqueous extract but not in ethanolic extract. Cardiac glycosides were observed in hexane and ethanolic extracts.

Phytochemical studies on *Ulva lactuca*

Among the five solvents used to extract the phytochemicals from *U. lactuca*, more number of metabolites was observed in the ethanolic extract (Table 1). Steroids, alkaloids, phenolics, saponins, cardiac glycosides and terpenoids were observed in the ethanolic extract of *U. lactuca*. However tannins were observed in hexane, petroleum ether, chloroform and aqueous extracts but not in ethanolic extract. Cardiac glycosides were observed in hexane, petroleum ether and ethanol but failed to show their presence in chloroform and aqueous. Steroids were observed in chloroform and ethanolic extracts.

DISCUSSION

The seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industries. The results of the phytochemical analysis of aqueous, ethanolic, chloroform, petroleum ether and hexane extracts of seven green seaweeds viz., *Ulva fasciata, Caulerpa scalpelliformis, Halimeda macroloba,, Enteromorpha compressa, Caulerpa corynephora* and *Ulva lactuca* revealed the presence of a good number of secondary metabolites.

Among the five solvents tested, the good number of metabolites was observed in ethanolic extracts of the selected green seaweeds. Similar to the present observation Ganga Rao Battu *et al.*, (2011) also observed maximum number of metabolites presence in the ethanolic extracts of seaweeds. Sumathi and Krishnaveni (2012) also observed steroids, alkaloids, tannins, saponins and cardioactive glycosides presence in the methanolic extracts of *Caulerpa scalpelliformis* and *Chaetomorpha antennia.* Johnson *et al*., (2012) and Jeeva *et al*., (2012) also noticed the maximum number of metabolites occurrence in the methnaolic extracts of *Sargassum wightii* and *Ulva reticulata* respectively.

Alkaloids rank among the most efficient and therapeutically significant plant substances (Okwu, 2005). They comprise the largest single class of secondary plant substances containing 5,500 alkaloids (Harborne, 1973). Furthermore, alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs (Harborne, 1973; Okwu, 2005). Alkaloids can work on the nervous system of the human body and be used for analgesic, antispasmodic and bacterial effects (Okwu and Josiah, 2006). The presence of alkaloids in the present study was confirmed in ethanolic extracts of *Ulva fasciata*, *Caulerpa scalpelliformis, Halimeda macroloba*, *Caulerpa corynephora* and *Ulva lactuca*. In *Enteromorpha compressa* alkaloids confirmed its presence in petroleum ether extract and this augments the use of alkaloid in global pharmaceutical market.

Phenolics are one of the most ubiquitous groups of secondary metabolites found throughout the plant kingdom (Boudet, 2007). Tannins, flavonoids, glycosides and anthraquinone are the derivatives of phenolic groups. Phenolic compounds are commonly found in plants, including seaweeds and have been reported to have a wide range of biological activities including antioxidant properties (Yanishlieva-Maslarova, 2001; Nagai and Yukimoto, 2003; Athukorala *et al*., 2003; Heo *et al*., 2005; Duan *et al*., 2006; Kuda *et al*., 2007; Zheng *et al.*, 2008 and Wang *et al*., 2009), antibacterial, antiviral, antifungal (Adekunle and Ikumapayi, 2006) and antiulcer (Kolodziej and Kiderlen, 2006). The phenolic groups' presence was occurred in ethanolic extracts of *Caulerpa corynephora*, *Caulerpa scalpelliformis*, *Chaetomorpha antennia, Enteromorpha compressa, Halimeda macroloba, Ulva fasciata* and *Ulva lactuca*.

Terpenoids are secondary plant metabolites that occur in a wide range of plant species (Hostettmann and Marston, 1995). Saponins and steroids are the derivatives of terpenoids. They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack (Osbourn, 1996). The natural role of saponins in plants is thought to be protection against attack by pathogens (Morrissey and Osbourn, 1999). These molecules also have considerable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics (Hostettmann and Marston, 1995).

Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, antiinflammatory, anti-feedent and hemolytic effects (Xu *et al*., 1996). In the present investigation also, saponins revealed their presence in ethanolic extracts of *Ulva fasciata, Halimeda macroloba* and *Ulva lactuca*. In addition to that, petroleum ether extracts of *Enteromorpha compressa* showed the presence of saponins. In the present study, steroid was present in various extracts of *Caulerpa corynephora, Caulerpa scalpelliformis, Enteromorpha compressa, Halimeda macroloba, Ulva fasciata* and *Ulva lactuca*.

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Table 1: Phytoprofile of Green Seaweeds

Seaweeds	*C. cornyephora*					*C. scalpelliformis*					*C. antennia*					*E. compressa*					*H. macroloba*					*U. fasciata*					*U. lactuca*				
Metabolites	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**	**H**	**P**	**C**	**E**	**A**
Steroids	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-
Alkaloids	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-
Phenolics	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-
Flavonoids	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-		-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-		+	-
Tannins	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+
Amino acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
Cardiac glycosides	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	+	-	+	-

H – Hexane; P – Petroleum Ether; C- Chloroform; E – Ethanol; A – Aqueous / Water.
+/- - indicates the presence / absence of metabolites.