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PHYTOCHEMICAL STUDIES ON *STOECHOSPERMUM MARGINATUM* (AG.) KUETZ USING UV-VIS AND HPLC PROFILE

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ABSTRACT

The present study was aimed to explore phytochemical constituents present in *Stoechospermum marginatum* (Ag.) Kuetz and to evolve the biochemical markers for the economically important *S. marginatum*. The extracts of *S. marginatum* were scanned in the wavelength ranging from 200 - 1100 nm by using Shimadzu Spectrophotometer. HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, which was equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. Out of 156 (2 x 6 x 13 = 156) qualitative phytochemical tests for the presence or absence of the metabolites, 54 qualitative tests showed positive results (presence of metabolites in the extracts) and the remaining 102 tests failed to show the presence of metabolites. The 54 positive results explained the presence of alkaloids, steroids, phenolic groups, saponins, tannins, flavonoids, glycosides, proteins and sugars. The results of ash analysis discovered the existence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *S. marginatum*. The UV-VIS profile of petroleum ether, aqueous, benzene, methanolic and isopropanol extracts of *S. marginatum* confirmed the variety of metabolites presence. The HPLC profile of *S. marginatum* aqueous extracts showed three prominent peaks at a retention time of 1.993, 2.177 and 2.683. The cold methanolic extracts of *S. marginatum* profile displayed one prominent peak at a retention time of 2.660 min and some moderate peaks are also observed at a retention time 2.007 min, 2.157 min, 2.467 min and 4.050 min.

KEYWORDS: Phytochemistry; UV-VIS; HPLC; *Stoechospermum marginatum*; Seaweed

INTRODUCTION

Marine algae are the excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals (Viron et al. 2000, Sanchez-Machado et al. 2004, Fayaz et al. 2005). Seaweeds are suitable for human and animal feed, as well as for fertilizer, fungicides, herbicides and phyco-colloids. 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 (Oh et al. 2008, Gopala et al. 2007). Bio-stimulant properties of seaweeds are explored for use in agriculture and antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anti-coagulants, anti-ulcer products and suspending agents in radiological preparations. Compounds with cytostatic, antiviral, anthelmintic, antifungal, antibacterial and antioxidant activities have been detected in green, brown and red algae (Lindequist and Schweder 2001; Newman et al. 2003). Many of these compounds are bioactive and have been extensively studied using bioassays and pharmacological assays (Paul and Fenical 1987). So far, many chemically unique compounds of marine origin with different biological activities have been isolated and a number of them are under investigation or are being developed as new pharmaceuticals (Faulkner 2000; Da Rocha et al. 2001; Schwartsmann et al. 2001). For centuries, many of the seaweed secondary metabolites (SSM) have been used for traditional medicines due to their therapeutic potentials (Fitton 2006). So far, more than 2400 SSM are described and many of them are natural blueprints for the development of new drugs (Al-Fadhli et al. 2006; El-Baroty et al. 2007). 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. A number of studies on antimicrobial and pharmacological activity of solvent extracts from marine algae are reported (Thirumaran et al. 2006; Thirumaran and Anantharaman 2006 a; Salvador et al. 2007; Shanmughapriya et al. 2008; Manilal et al. 2009; Eluvakkal et al. 2010; Manivannan et al., 2011). But only few reports are available on the phytochemical studies (UV-VIS and HPLC) on the seaweeds from Gulf of Mannar and Peninsular coast of India (Johnson and Krishnaveni 2011; Johnson and Essakiammal devi 2011). With this background, the present study was aimed to explore the phytochemical constituents, to build up standard method for extraction, resolve the phytochemical profile and illustrate the physico-chemical characters viz., fluorescence, extractive, ash values of *Stoechospermum marginatum* (Ag.) Kuetz.

MATERIALS AND METHODS

Stoechospermum marginatum (Ag.) Kuetz. were collected by handpicking from the coast of Rasthacaud (Lat N $08^{0}08'308''$ $E77^{0}32'80''$) Kanyakumari District, Tamil Nadu, India. 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 at room temperature in shade. Shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use. To compare the hot and cold extraction, the dried and powered materials (5 g) were extracted successively with 250 ml of petroleum ether, methanol, chloroform, acetone, benzene, isopropanol and water using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman 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. For cold extraction, 2 g of air dried powder of sample was extracted with 50 ml of solvents viz., ethanol, acetone, petroleum ether, chloroform, benzene and water for 72 h. 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, triterpenoids, reducing sugars, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids, protein, glycosides and anthroquinones. Phytochemical screening of the extracts was carried out according to the standard methods (Harborne 1998). For the proximate analysis, the extracts were examined under visible and UV light. These powdered materials were also treated with various reagents such as 50% nitric acid, acetone, ethanol, 50% sulphuric acid, 1N HCL and 1N NaOH and changes in colour were recorded (The Pharmacopoeia of India 1996). For UV-VIS spectrophotometer and HPLC analysis, the extract was centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No. 1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. To detect the UV-VIS spectrum profile of the crude extracts of *S. marginatum*, the extracts were scanned in the wavelength ranging from 200 - 1100 nm by using Shimadzu Spectrophotometer and the characteristic peaks were detected. The qualitative UV-VIS fingerprint profile of benzene, chloroform and methanolic extracts of *S. marginatum* was selected at a wavelength of 300-700 nm due to the

sharpness of the peaks and proper baseline. HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil ® BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC-10 AT *VP* pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), CTO- 10AS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and reverse phase Luna 5°C18 (2) Phenomenex column (250mm X 4.6mm) was used. The mobile phase components methanol: water (45:55) were filtered through 0.2 µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270 kgf / cm^2. The column temperature was maintained at 27°C. 20µl of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton). The elution was carried out with gradient solvent systems with a flow rate of 1 ml min-1 at ambient temperature (25-28°C). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µl while the wavelength of the UV-Vis detector was set at 254 nm (Sharanabasappa et al. 2007; Mallikharjuna et al. 2007).

RESULTS

Thus out of (2 x 6 x 13 = 156) 156 preliminary phytochemical tests for the presence or absence of compounds, 54 qualitative tests showed positive results (presence of metabolites in the extracts) and the remaining 102 tests failed to show the presence of metabolites. The 54 positive results explain the presence of alkaloids, steroids, phenolic groups, saponins, tannins, flavonoids, glycosides, proteins and sugars. Xanthoproteins, quinones, coumarins and catechin failed to express their presence in any of the six extracts of *S. marginatum* assessed. The cold extracts of *S. marginatum* showed the maximum presence of phenolics and sugars in four different extracts followed by proteins, steroids, alkaloids and glycosides in 3 extracts, saponins and tannins in 2 different extracts. Among the six different extracts, isopropanol extract show the presence of maximum number (6) of compounds. Benzene, petroleum ether, methanolic and aqueous extracts of *S. marginatum* revealed the presence of four metabolites and 3 metabolites presence were detected in the crude chloroform extract of *S. marginatum* (Table 1). The hot extracts of *S. marginatum* demonstrate the maximum existence of sugars in five different extracts followed by phenols, alkaloids, steroids and glycosides in four extracts, tannins and

saponins in two different extracts. Among the six different hot extracts, petroleum ether extract displayed the presence of maximum number (7) of metabolites. Next to that, isopropanol extract illustrated the presence of six metabolites, followed by benzene extract with five metabolites and aqueous and methanol extract shows 4 metabolites each (Table 1). The results of the ash analysis revealed the presence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *S. marginatum*. The fluorescence analysis of *S. marginatum* extracts are recorded in Table 2. The profile of petroleum ether extracts of *S. marginatum* showed the compounds separated at the nm of 926, 668, 610, 534, 504 and 324 with the absorption 0.006, 0.263, 0.04, 0.052, 0.071 and 3.675 respectively. The aqueous extract profile of *S. marginatum* showed the compounds separated at the nm of 910, 664, 606, 536 and 324 with the absorption 0.06, 1.849, 0.59, 0.648 and 1.604 respectively. The benzene extract of *S. marginatum* profile showed the compounds separated at the nm of 668, 612, 536, 504, 414 and 408 with the absorption 0.365, 0.057, 0.071, 0.107, 0.891and 0.619 respectively. The methanolic extract of *S. marginatum* profile showed the compounds separated at the nm of 664, 606, 536, 500 and 408 with the absorption 0.33, 0.099, 0.118, 0.16 and 1.273 respectively. The isopropanolic extract of *S. marginatum* profile showed the compounds separated at the nm of 664, 608, 536, 502, 406 and 284 with the absorption 0.172, 0.051, 0.051, 0.063, 0.522 and 2.048 respectively.

Table -1 Preliminary Phytochemical Studies on *Stoechospermum marginatum* (Ag.) Kuetz.,

Compounds	*S. marginatum* - ***Sox***						*S. marginatum* - ***Cold***					
	C	M	I	B	Aq	P	C	M	I	B	Aq	P
Alkaloids	+		+		+	+	+		+		+	
Phenols	+	+		+		+	+	+		+		+
Flavonoids		+						+				
Saponins				+		+				+		+
Proteins		+	+			+		+	+			+
Triterpenoids	+	+					+	+				
Steroids			+	+	+	+			+		+	+
Tannins			+		+				+		+	
Xanthoprotiens												

Quinnone												
Catechin												
Glycosides		+	+	+		+		+	+	+		
Coumarins												
Sugars	+		+	+	+	+	+		+	+	+	

Table 2: Proximate Analysis of *S. marginatum*

Solvents	***S. marginatum***	
	Ordinary light	**UV light**
Chloroform	Light green	Light green
Benzene	Yellowish green	Fluorescent green
Methanol	Green	Green
Aqueous	Yellow	Light green
50% H_2SO_4	Light green	
1N HCl	Light green	
Ethanol	Yellowish green	
NaOH	Greenish blue	
HNO_3	Greenish yellow	

The qualitative HPLC fingerprint profile of aqueous and methanolic extracts of *S. marginatum* was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. Aqueous extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *S. marginatum*. Three compounds were separated at different retention time 1.993, 2.177 and 2.683 respectively (Fig. 1A). The cold methanolic extracts of *S. marginatum* profile displayed one prominent peak at a retention time of 2.660 min and some moderate peaks also observed at a retention time 2.007 min, 2.157 min, 2.467 min and 4.050 min (Fig. 1B).

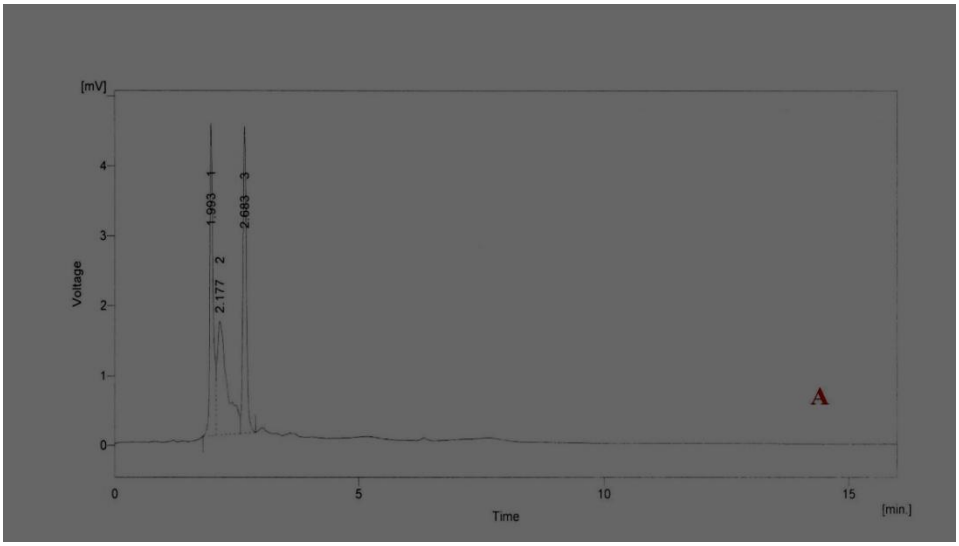

Fig. 1 A: HPLC fingerprint profile of aqueous extracts of *S. marginatum*

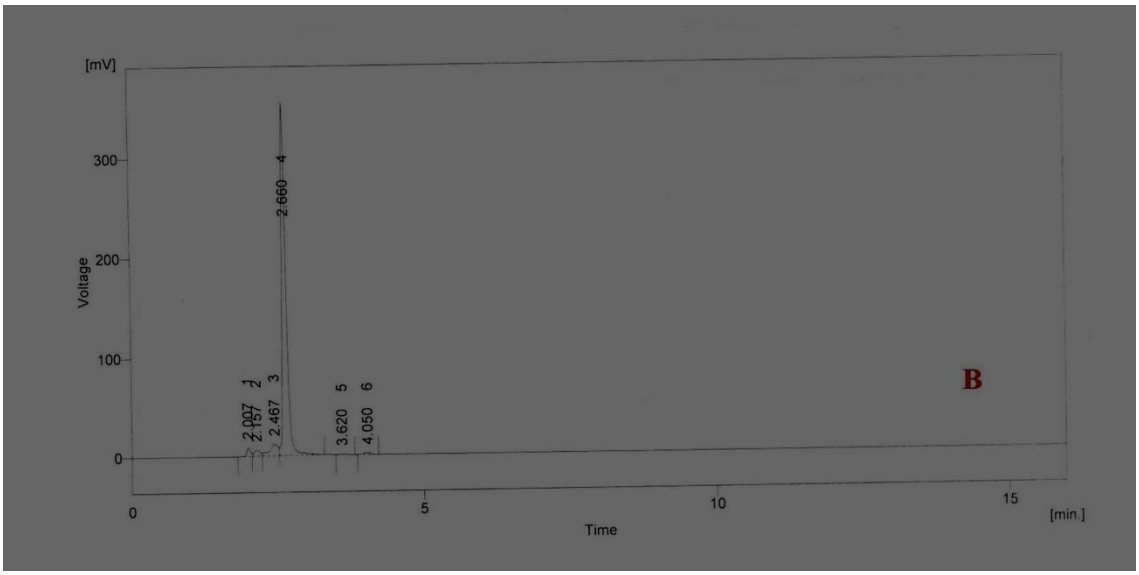

Fig. 1 B: HPLC fingerprint profile of methanolic extracts of *S. marginatum*

DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, large number of plants has been widely used for the treatment of various diseases due to their antioxidant properties. The inhibitory substances biosynthesized by the seaweeds were noted earlier in 1917 (Harder and Oppermann 1953). The first observation regarding antibiotic activities of seaweeds was reported in 1944 (Pratt et al. 1944). Recent findings evidenced that

seaweeds contain antibacterial (Tuney et al. 2006), antiviral (Garg et al. 1992; Serkedjieva 2004), antifungal (Aliya and Shamaeel 1999; Tang et al. 2002), cytotoxic (Smith 2004) and larvicidal potentials (Thangam and Kathiresan 1991). The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower form of animals (Smith 2004). The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments. Thus, marine algae are one of the richest sources of known and novel bioactive compounds (Faulkner 2002; Blunt et al. 2007). The results of the phytochemical analysis of various solvent extracts revealed the presence of primary and secondary metabolites with varied degree.

The preliminary phytochemical screening clearly tallies with the colour of the extracts (fluorescence) 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. The seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins and steroids which have been extensively used in the drug and pharmaceutical industry. Tannins are used in pharmaceutical preparations because of their astringent action. Tannins are known to possess general antiparasitic effects (Kolodziej and Kiderlen 2005), antiviral (Lu et al. 2004), antibacterial (Akiyama et al. 2001) antioxidant activities (Riviere et al. 2009). At low concentration, tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism (Adekunle and Ikumapayi 2006). Recent reports show that tannins may have potential value as cytotoxic and anti-neoplastic agents (Aguinaldo et al. 2005). Aside from the use of tannins as antimicrobial agents or prevention of dental caries, they are now being used in the manufacture of plastics, paints, ceramics and water softening agents (Bandarayanake 2002). Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications (Kolodziej and Kiderlen 2005). Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially polyphenols have antioxidant activity (Duan et al. 2006; Chandini et al.

2008; Ganesan et al. 2008; Wang et al. 2009). The major active phenolic compounds in different seaweed extracts have been reported to be phlorotannins and fucoxanthin (Yan et al. 1999). Flavonoids are known as nature's tender drug which possesses numerous biological and pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcenogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants. Of these biological activities, the anti-inflammatory capacity of flavonoids has long been utilized in Chinese medicine and in the cosmetic industry as a form of crude plant extracts (Kim et al. 2004; Moon et al. 2006; Veitch 2007; Jiang et al. 2008; Wu et al. 2008).

Hordenine was the first alkaloid isolated from a marine algae in 1969 (Guven et al. 1969; Guvan et al. 1970). Polyethylamine (PEA) alkaloids in the human brain acts as neuromodulator and neurotransmitter. PEA isolated from seaweeds has been shown to relieve depression in 60% of depressed patients (Barroso and Rodriguez 1996). Substituted PEAs are pharmacologically active compounds as hormones, stimulants, hallucinogens, entactogenes, anorectics, bronchodilators and antidepressants (Saavedra 1978). Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects (Xu et al. 2000). Marine algae have shown to be good source of unsaponofiable, non toxic sterols that have medicinal value (Orcuut 1970). Cholesterol has been found to be present at high concentrations in Caribbean red seaweeds (Govindan and Hodge 1993). Cholesterol is the predominant sterol of red algae and fucosterol in brown algae (Combaut et al. 1985). Steroids exhibit high cytotoxic activity towards human adenocarcinoma cells and T-lymphocyte leukemia cells (Iwashima et al. 2001). The presence of flavonoids, steroids, phenolics, saponins, tannins, glycosides in the crude extracts of *S. marginatum* suggest that seaweeds can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcenogenic and anti-ulcer agents in the near future. In addition, they also possess hepatoprotective properties and cytotoxic activities. In the present investigation, the extractive values for *S. marginatum* were produced. These are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in a particular solvent. In addition, the behaviour of the drug powder with different chemical reagents was also produced. It will be helpful in characterization of the crude

drug and to identify and classify the seaweeds.

HPLC identification test are required to confirm the presence of the active constituents and potential adulterant in ayurvedic drugs. In the present study, the UV-VIS and HPLC profile for *S. marginatum* was evolved. Thus the present studies on *S. marginatum* exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. Fluorescence, UV-VIS spectroscopic and HPLC profiles can be used as biochemical markers in the pharmaceutical industries to identify the authentic mother plants and differentiate its adulterants.

CONCLUSION

The above mentioned results show that *S. marginatum* contain rich sources of phytoconstituents particularly flavonoids, steroids, tannins, alkaloids, phenols and glycosides which can be isolated and further screened for different kinds of biological activities depending on their reported therapeutic uses. Quantitative analysis of these phytochemicals may also be done to guide the researchers on which particular bioactive class of compounds may be subjected to subsequent target isolation.

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