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ORIGINAL ARTICLE

UV–VIS and HPLC studies on *Amphiroa anceps* (Lamarck) Decaisne

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Abstract The present study was aimed to explore phytochemical constituents present in *Amphiroa anceps* (Lamarck) Decaisne. The extracts of *A. anceps* were scanned in the wavelength ranging from 200 to 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 ($2 \times 6 \times 13 = 156$) tests for the presence or absence of the above compounds, 42 tests gave positive results and the remaining 114 showed negative results. The 42 positive results showed the presence of alkaloids, steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids, glycosides, proteins and sugars. The results of the ash analysis discovered the existence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *A. anceps*. The UV–VIS profile of *A. anceps* benzene extracts showed peaks at 670, 612, 536, 504, and 412 nm with the absorption 2.004, 0.333, 0.417, 0.608 and 3.311. The UV–VIS profile *of A. anceps* chloroform extracts showed the peaks at 666, 608, 538, 502 and 416 nm with the absorption 1.029, 0.39, 0.458, 0.552 and 2.648. The qualitative UV–VIS spectrum profile of *A. anceps* methanolic extracts showed peaks at 656 and 330 nm with the absorption 0.295 and 3.656. The HPLC profile of *A. anceps* aqueous extracts showed two prominent peaks at a retention time of 1.737 and 2.680 min and some moderate peaks were observed with the retention time 4.083, 6.387 and 1.490 min. The qualitative HPLC fingerprint profile of isopropanol extract of *A. anceps* showed one prominent peak at a retention time of 2.673 min. The HPLC profile of *A. anceps* methanolic extracts illustrated

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two prominent peaks at a retention time of 1.927 and 2.667 min and some moderate peaks were also observed with the retention time 2.347, 4.077 and 5.873 min, respectively.

1. Introduction

Seaweeds are marine plants, constituting important renewable marine resources; these grow in the shallow waters of sea wherever suitable substrata are available. In India seaweeds are found more abundantly along the southeastern and northeastern parts of the coast. There are 681 or so known species of seaweeds in India, of which 60 are commercially important. Historically seaweeds provide essential economic, environmental, aesthetic, and cultural benefits to humanity (Dhargalkar and Neelam, 2005). For centuries, many of the seaweed secondary metabolites (SSM) have been used in traditional medicines due to their therapeutic potentials (Fitton, 2006). Fresh and dry seaweeds are extensively consumed as food and medicine 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 essential for human nutrition (Fayaz et al., 2005). Seaweeds have some of the valuable medicinal compounds such as antibiotics, laxatives, anticoagulants and anti-ulcer products. 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, 2000a,b; DaRocha et al., 2001; Schwartsmann et al., 2001). So far, more than 2400 SSM have been described and many of the SSM are natural blueprints for the development of new drugs (Al-Fadhli et al., 2006; El-Baroty et al., 2007). Marine algae are continuously exposed to many biotic and abiotic pressures which influence the organism's physiology, which in turn leads to the production of multifunctional natural secondary metabolites.

Several of these compounds are constitutive, existing in biologically active forms in healthy seaweeds. The major secondary metabolites produced by seaweeds are halogenated compounds displaying anti-bacterial, anti-fungal, anti-viral, anti-fouling and anti-feedent properties (Blunt et al., 2007). Although thousands of bioactive compounds have been discovered, today there is a need for the discovery of novel therapeutic compounds due to the origin of several new pathogenic diseases and resistant strains of pathogens. A number of studies on antimicrobial and pharmacological activities of different solvent extracts from marine algae are reported (Thirumaran and Anantharaman, 2006; Thirumaran et al., 2006; Salvador et al., 2007; Shanmughapriya et al., 2008; Manilal et al., 2009; Manivannan et al., 2011; Eluvakkal et al., 2010). But only few reports are available on intensive phytochemical studies, particularly on UV–VIS and HPLC studies, on the seaweeds from Gulf of Mannar and the Peninsular coast of India. With this background, the present study was aimed to explore the phytochemical constituents of the petroleum ether, benzene, chloroform, isopropanol, methanolic and aqueous, extracts of red seaweed, *Amphiroa anceps* (Lamarck) Decaisne by UV–VIS and HPLC.

2. Materials and methods

Amphiroa anceps (Lamarck) Decaisne were collected by hand-picking from the coast of Vattakottai, 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 by using tissue blender. The powdered samples were stored in a 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 by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whattman filter paper (No. 1) and then concentrated in vacuum at 40 °C using Rotary evaporator. The residues obtained were stored in a freezer at −20° C until further tests. Two grams of the 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 (cold crude extracts). The different extracts were examined for the presence of steroids, triterpenoids, reducing sugars, phenolic compounds, saponins, xanthoproteins, tannins, flavonoids, proteins, 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, 1 N HCl and 1 N 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 *A. anceps*, the extracts were scanned in the wavelength ranging from 200 to 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 *A. anceps* were 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

an 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 (250 mm × 4.6 mm) 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 1 ml/min which yielded a column backup pressure of 260–270 kgf/cm^2. The column temperature was maintained at 27 °C. Twenty microliters of the 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 an 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 whilst the wavelength of the UV–VIS detector was set at 254 nm (Sharanabasappa et al., 2007; Mallikharjuna et al., 2007).

3. Results

By preliminary phytochemical screening thirteen different chemical compounds (steroids, alkaloids, phenolic groups, saponins, tannin, flavonoids, anthraquinone, carbohydrates, carboxylic acid, coumarins, proteins, sugars and xanthoproteins) were assessed in six different extracts of *A. anceps*. Thus out of (2 × 6 × 13 = 156) tests for the presence or absence of the above compounds, 42 tests gave positive results and the remaining 114 showed negative results. The 42 positive results showed the presence of alkaloids, steroids, phenolic groups, saponins, tannin, flavonoids, terpenoids, glycosides, proteins and sugars. Xanthoproteins, coumarins and catechin failed to show their presence in any of the six extracts of *A. anceps*. In the soxhlet extracts of *A. anceps* phenol showed the maximum presence in ten different extracts followed by sugar in eight extracts alkaloid, steroid and tannin in six different extracts. Among the twelve different extracts, aqueous extracts both cold and soxhlet, demonstrated the presence of maximum number (**6**) of compounds. Isopropanol and petroleum ether extracts showed the presence of four compounds. Three compounds were identified in chloroform and benzene extracts and only one compound was present in methanol extract of *A. anceps* (Table 1). The results of ash analysis showed the presence of sulphur, calcium, magnesium, iron, phosphorous and chlorine in all the extracts of *A. anceps*.

The profile displayed the compounds separated at the nm of 670, 612, 536, 504 and 412 with the absorption 2.004, 0.333, 0.417, 0.608 and 3.311, respectively (Fig. 1, Table 2). The profile showed the compounds separated at the nanometer of 666, 608, 538, 502 and 416 with the absorption 1.029, 0.39, 0.458, 0.552 and 2.648, respectively (Fig. 1; Table 2). The profile showed the compounds separated at the nm of 656 and 330 with the absorption 0.295 and 3.656, respectively (Fig. 1; Table 2).

The qualitative HPLC fingerprint profile of aqueous, isopropanol and methanol extracts of *A. anceps* was selected at a wavelength of 254 nm due to the sharpness of the peaks and proper baseline. The aqueous extract of *A. anceps* prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in *A. anceps*. Two compounds were separated at different retention times of 1.737 and 2.680 min (Fig. 2). The HPLC profile of *A. anceps* isopropanol extract displayed one prominent peak at a retention time of 2.673 min (Fig. 3). The HPLC profile of *A. anceps* methanolic extracts (cold extract) displayed two prominent peaks at retention times of 1.927 and 2.667 min and some moderate peaks were also observed at retention times of 2.347, 4.077 min and 5.873 min (Fig. 4).

4. Discussion

Very different kinds of substances have been obtained from marine organisms because they live in a very exigent, competitive, and aggressive environment which demands the production of quite specific and potent active molecules. In the present study alkaloids have been observed in both cold and hot extracts of *A. anceps*. Omulokoli et al. (1997) and Cowan (1999) confirmed the antimicrobial properties of alkaloids in plants. It suggests that the aqueous, chloroform and isopropanol

Table 1 Preliminary phytochemical studies on *Amphiroa anceps* (Lamarck) Decaisne.

Compounds	Cold						Soxhlet						Tot
	C	M	I	B	Aq	P	C	M	I	B	Aq	P	
Alkaloids	+		+		+		+		+		+		06
Phenols	+	+		+	+	+	+	+		+	+	+	10
Flavonoids													00
Saponins				+		+				+		+	04
Proteins													00
Quinones													00
Steroids			+		+	+			+		+	+	06
Tannins			+		+	+			+		+	+	06
Xanthoproteins													00
Catechin													00
Glycosides					+						+		02
Coumarins													00
Sugars	+		+	+	+		+		+	+	+		08
Total	3	1	4	3	6	4	3	1	4	3	6	4	42

C – Chloroform; B – Benzene; Aq – Aqueous; M – Methanol; I – Isopropanol; P – Petroleum ether.

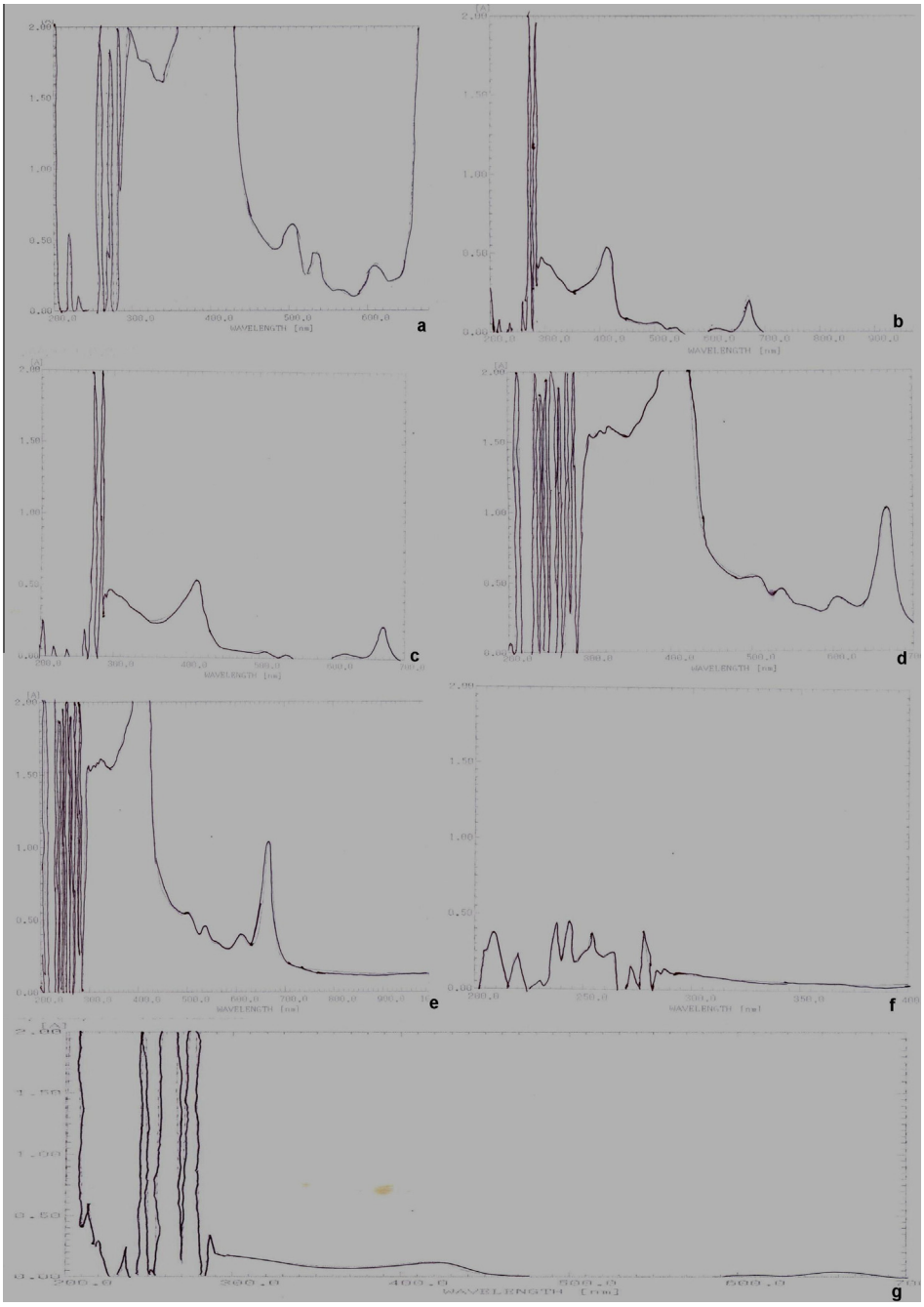

Figure 1 UV–VIS spectrum of *Amphiroa anceps*. (a) Benzene cold extract; (b) benzene cold extract; (c) benzene cold extract; (d) chloroform cold extract; (e) chloroform cold extract; (f) petroleum ether cold extract; (g) isopropanol soxhlet extract.

Table 2 UV–VIS peak values of different extracts of *Amphiroa anceps* (Lamarck) Decaisne.

Solvents	Chloroform		Methanol		Benzene	
S. No.	λ	Abs	λ	Abs	λ	Abs
1	666	1.029	656	0.295	670	2.004
2	608	0.39	330	3.656	612	0.333
3	538	0.458			536	0.417
4	502	0.552			504	0.608
5	416	2.648			412	3.311

extracts of *A. anceps* with alkaloid, can be used as antimicrobial agents in the pharmaceutical industry. In the meantime, seaweed extracts are considered to be a rich source of phenolic compounds (Athukorala et al., 2003; Heo et al., 2005). In the present study also the presence of phenolics was confirmed by the qualitative and quantitative analysis in the crude extracts of the selected seaweed. 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). Reports have revealed that phenolic compounds are one of the most effective antioxidants

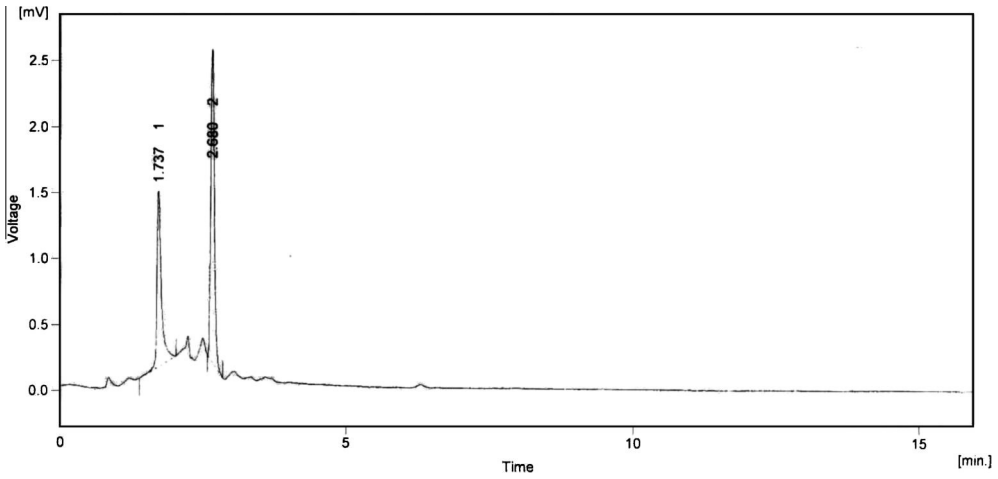

Figure 2 HPLC profiles of *Amphiroa anceps* – aqueous cold extract.

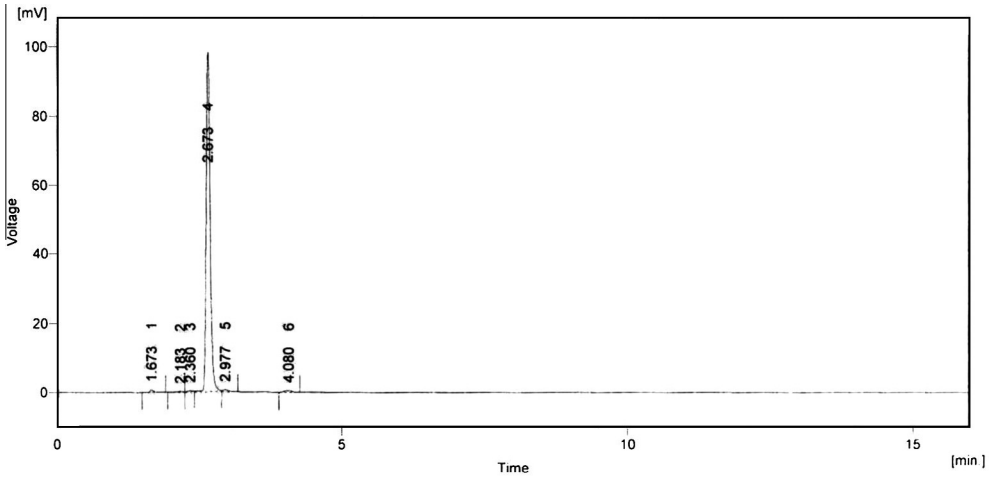

Figure 3 HPLC profiles of *Amphiroa anceps* – isopropanol soxhlet extract.

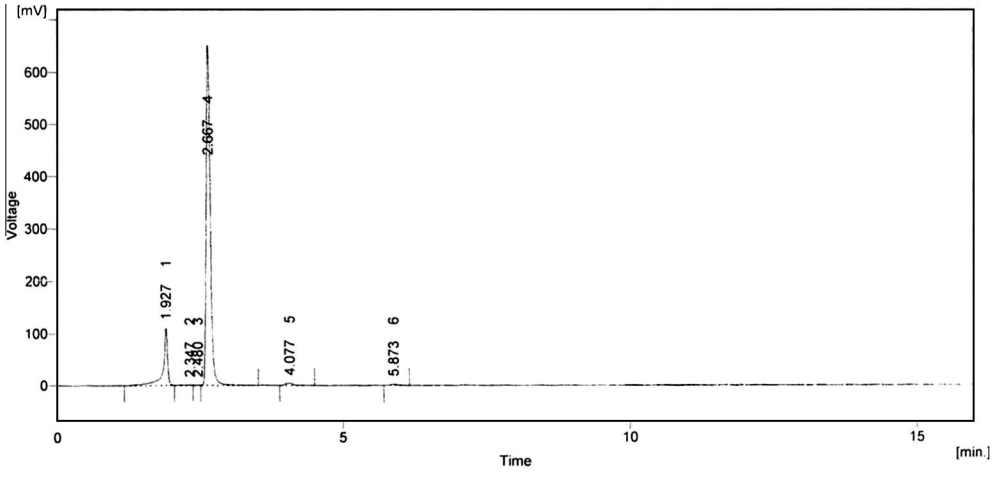

Figure 4 HPLC profiles of *Amphiroa anceps* – methanol cold extract.

(Nagai and Yukimoto, 2003). The results of the present study show that algal polyphenolic compounds present in *A. anceps* are an effective source of antioxidants, therefore the seaweed extracts could have potential applications in food industries (Yan et al., 1996). Tannins are widespread among terrestrial and marine plants (Haslam, 1989; Waterman and Mole, 1994;

Huang et al., 2008). In contrast to terrestrial tannins, phlorotannins are tannin compounds which have been found only in marine algae. Phlorotannins purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton. Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavengers than other polyphenols derived from terrestrial plants, including green tea catechins, which only have three to four rings (Hemat, 2007). Many tannin-containing drugs are used in medicine as astringents. 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 antidotes. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications (Lü et al., 2004; Kolodziej and Kiderlen, 2005). The present study confirms the presence of tannin in all the extracts of *A. anceps*. It suggests that the selected seaweeds can be used as antiviral, antibacterial, antiparasitic agents and exercised to treat diseases like ulcer, gonorrhoea, leucorrhoea etc.

Saponins are considered 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 haemolytic effects (Xu et al., 1996; George et al., 2002). These observations cited on phytochemical compounds support our findings on the usefulness of seaweeds in traditional medicament. The plants known as medicinal, are rich in secondary metabolites that include alkaloids, glycosides, flavonoids, insecticides, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of plants across the world. In the present investigation the seaweed *A. anceps* has been selected from India for phytochemical screening. The present phytochemical study revealed the presence of phenols, alkaloids, tannins, steroids, glycosides, saponin and flavonoids in all seaweed extracts with varying degrees. Many workers revealed that the crude extracts of Indian seaweeds are active against Gram-positive bacteria (Rao and Parekh, 1981). The present study confirms that cold extraction method will be useful to extract maximum secondary number of secondary metabolites from *A. anceps*. It is proved by the presence of 23 different types of compounds in cold extract in contrast to the presence of only 19 compounds in the hot extract of *A. anceps*. The antimicrobial and pharmacological activity of the selected seaweed, *A. anceps*, may be due to one/more groups of the above phyto-constituents.

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