COMPLETE PHARMACOGNOSTICAL STUDY ON RED SEAWEED *PORTERIA HORNEMANNII* (LYNGBYE) P.C.SILVA

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ABSTRACT

As marine algae have high potential in the pharmaceutical field and its importance made this study to evaluate the pharmacognostic, and to conduct the preliminary phytochemical, biochemical and inorganic mineral analysis of the successive extract of a red seaweed *Portieria hornemannii*. It was concluded from the study, that the Marine algae are the good source of unsaponifiable non-toxic steroids and other secondary metabolites that proved the evidence of pharmacognostic standards has been exposed to highlight biology importance in pharmaceutical line. The present result would be of help to isolate and characterize the diverse pharmacologically active compounds and importance supporting their varied biological activities and the medicinal values.

KEYWORDS: *Portieria hornemannii*, macroscopy, microscopy, pharmacognostic studies, phytochemical analysis, GCMS.

INTRODUCTION

Seaweeds are important renewable marine resource of the coastal waters. Fresh and dry seaweeds are traditionally consumed as a sea vegetable in many countries and their safety has been recognized (Manivannan *et al.,* 2011). They are of high nutritional value (Jimenez Escrig & Goni Cambrodon, 1999) and the quality of proteins and lipids are comparatively

better than other vegetables (Dawes, 1998). Red and brown algae are mainly used as human food sources and serve as an important source of bioactive substances. With the crude and purified compounds obtained from seaweeds several bioactivity were carried out by researchers (Fuller *et al.,* 1992) and are one of the chief biological agents that have been studied for the various biological activities.

From a nutritional standpoint, seaweeds comprises of high minerals such as iodine and calcium, soluble dietary fibres, vitamin B12 and specific phytochemicals such as fucoxanthin, fucosterol, phlorotannin (Bocanegra *et al.,* 2009). Apart from these components, seaweeds consist of antibiotics, laxative, anticoagulant, anti-ulcer agents. Although seaweeds present such a wide range of bioactive molecules in them, they are still under-exploited materials. Further research in these components might be helpful in utilizing seaweeds in neutraceutical and pharmaceutical industries (Pereira and Leonel, 2011).

Different authors have pointed out that the chemical composition of seaweeds varies with species, habits, maturity and their environmental conditions (Sanchez-Machado, Lopez –Cervantes, & Lopez-Hernandez, 2004). The extracts from various *brown algae* have been used as a traditional medicine in Asia (Anastyuk *et al.,* 2017). The bioactive compounds present in extract such as carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals (Fleurence, 1999; Bhaskar *et al.,* 2004)) present in the extracts of the red seaweeds are primarily used for treating diseases like cancer, Acquired immune deficiency Syndrome (AIDS), arthritis, etc.

The tropical red alga *P. hornemannii* (Lyngbye) Silva (*Gigartinales, Rhizophyllidaceae*) can be found in a variety of high wave energy habitats like the ones found in the coastal region of Mandapam, Rameshwaram that has several acyclic monoterpenes as minor metabolites (Paul *et al.,* 1987). Feeding assays on the coastal region have shown

ochtodene to be an effective feeding-deterrent to herbivorous reef fishes (Paul *et al.,* 1988, 1990, 1993). It proved to exhibit notable site-to-site variation in secondary metabolite production (Paul *et al.,* 1987; Fuller *et al.,* 1992, 1994; Puglisi & Paul, 1997). The major secondary metabolite of *P. hornemannii* is apakaochtodene B (Puglisi & Paul, 1997), which is an effective deterrent against herbivores (Paul *et al.,* 1987, 1990, 1992). Several other halogenated monoterpenes including apakaochtodene A are minor metabolites (Paul *et al.,* 1987; Puglisi & Paul, 1997). Puglisi and Paul (1997) showed that nutrient availability (i.e., nitrogen and phosphorus).

Phycoerythrin is a major pigment present in most of the red algae belonging to Phycobiliproteins. It is also used as a natural food dye Phycobiliproteins are largely appreciated for their immuno-enhancing, anti-inflammatory, anticarcinogenic, antioxidant and nutritive values (DeLange *et al.,* 1989). But still the basic study of the seaweed is unknown and their pharmacognostic studies remain without knowledge.

Hence the present study was aimed to investigate to determine the possible phytochemical components and for the first time the pharmacognostic study of red seaweed *P. hornemanii* (Lyngbye) P.C.Silva was also carried out which was collected from the Mandapam coastal region (Rameshwaram coast).

MATERIALS AND METHODS

Collection and Authentication

P. hornemannii (Lyngbye) P. C. Silva was collected from Mandapam, Gulf of Mannar (Lat.09º 17’N; Long.79º 08’E), Rameshwaram coast, Ramnad, South India, Tamil Nadu, and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, and India.

Processing of collected sample

The seaweeds collected were thoroughly rinsed with seawater to remove dirt and debris along with epiphytes, sand particles, and shells. Then, they were washed with running tap water followed by distilled water and dried in shade. The resulting dried material was coarsely powdered (passing through 40 size sieve) and utilized for further pharmacognostic and phytochemical studies.

Preparation of extract

In a round bottom flask, the powdered 50g of red algae was taken followed by the addition of 500 mL ethyl acetate and was subjecting evaporation and condensation process in a soxhlet apparatus at 64°C for 72 hours. the The extracts of the red seaweed, representing lower polar, polar and non-polar components were pooled together and evaporated under reduced pressure using rotary flash evaporator (Superfit, India). The crude extract was quantified and further analysis.

Macroscopic analysis

The external morphological properties like shape, colour, dimension, uprights or creeping and taxonomy of *P. hornemannii* was studied (Esau, 1964).

Microscopic analysis

Qualitative and quantitative microscopic evaluation was conducted on entire plant *Portieria hornemannii*. In this study transverse section of leaf (O'Brein *et al.*, 1964) were carried out using Toluidine blue O dye and the morphology was studied.

Physico-chemical constants

The physio-chemical analysis like the loss on drying, total ash , water soluble ash, acid soluble ash and sulphated ash determined according to the methods prescribed in Indian Pharmacopoeia (1996).

Phytochemical screening

Preliminary phytochemical screening of methanol extract of *P. hornemannii* was carried out to detect the phytochemical constitution of the extract using standard conventional methods (Harborne, 1998).

Biochemical analysis

The fresh plant was subjected to biochemical analysis of protein by Bradford's and Lowry method, carbohydrate by phenol-sulphuric acid method and lipid by Folch *et al*, (1957).

Inorganic mineral analysis

For the determination of mineral elements present in the methanolic extract of *P. hornemannii*, samples were dissolved in 1 M HNO_3 and H_2O_2 before being digested by a microwave. The concentration of the elements in the dried seaweed samples was determined by means of an atomic absorption spectrometer () equipped with a hollow cathode lamp according to the method described in MOOPAM (1989). The seaweed mineral concentrations were quantified from calibration curves of the respective standard elements.

Fluorescent analysis

Fluorescence analysis is one of the most important parameters for the evaluation of the quality, strength and purity of the selected plant material. Fluorescence analysis of dried and powdered seaweed was carried out according to the procedure described in Kirtikar *et al.*, (2005) by using the reagents and viewed in daylight and ultraviolet radiations. The colours

and fluorescence observed by application of different reagents in different radiations were recorded

Gas chromatography-mass spectroscopy (GC-MS)

The composition of the propitious algal extract was obtained using GC-MS (Perkin Elmer GC coupled to Clarus 680 turbo mass ver 5.4.2 with library version NIST-2008). The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the separation of components were done using Helium as carrier gas. Injector temperature was maintained at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 9 - 10 °C min^{-1} ; and 300 °C, where it was held for 5 - 6 min. The mass detector conditions were fixed with transfer line temperature of 240 °C; ion source temperature at 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

RESULTS

P. hornemannii is a red algal species which is abundant in the Mandapam coastal region is characterized by the presence Reddish orange colour of R-Phycoerythrin pigment as well as its pleasant fluorescence imparting variation among the other seaweeds.

The marine specimen *P. hornemannii* selected for the proposed work was collected from Mandapam coast, Rameshwaram, India and was authenticated. In order to study the therapeutic value of this species, Pharmacognostic parameters like microscopic observation, morphology, extractive values, and physicochemical parameters like a loss on drying, ash

values, inorganic constituents and mineral analysis were done based on the standard procedures available.

Macroscopic characters

The size of the Thalli is 15 - 20 cm tall, usually smaller; the colour of the seaweed is bright orange to red colour. The seaweed margin composed of several erect, overlapping flattened branches arising from small discoid holdfasts. Branching in one plane, irregularly pinnate-alternate forming rounded axils; the diameter of primary branches is 5 -7 mm; terminal branches at the distal portion of thalli with slightly expanded curved or in rolled tips; lower lateral branchlets with simple acute teeth.

Microscopic observation

Transverse section of *P. hornemannii* (Metcalfe and Chalk 1979)

Anatomy of thallus

The microscopic analysis showed the large-celled pseudo-parenchymatous medulla and cortex of smaller cells. The outer layer of cortex in parts of thalli contains many slightly larger gland cells (figure 1)

Figure 1: Microscopic structure of *P. hornemannii*

Yield of extraction

The percentage yield of extracts obtained from *P. hornemannii* using Methanol as a solvent was about 19 g from 100 g of powdered sample from the 72 hrs of extraction (Table 1).

Table 1: Shows the yield of extraction (*P. hornemannii*)

Parameters	
Solvent	Methanol
Amount of sample (g)	100 g
Boiling point	64.7° C
Total hours of extraction	72 hrs
Yield (g)	19 g
Colour of extract	Pale orange reddish colour with crystals

Physio-chemical constituents

The physio-chemical nature of *P. hornemannii* are shown in Table 2

Table 2: Physico-chemical analysis of *P. hornemannii*

Parameters	Values (%w/w)
total ash	12.5%
water soluble ash	4.98%
acid insoluble ash	7.51%
sulphated ash	0.39%
loss on drying	2.10%

All the parameters noted are pharmacognostic importance and utilized to differentiate between adulterant and original species.

Phytochemical and biochemical screening

Table 3 shows the presence of phytochemicals like tannins, alkaloids, steroids, phenolic compounds, terpenoids in the extract. The seaweed *P. hornemannii* was rich biochemical compounds such as lipid, protein and carbohydrate content.

Table 3: Phytochemical screening of methanol extracts of *P. hornemannii*

S.No	Phytochemicals	Methanol extract
1	Alkaloids	+
2	Anthroquinones	+
3	Phlobatannins	+
4	Cardiac Glycosides	+
5	Glycosides	-
6	Phenolic compounds	+
7	Flavonoids	+
8	Terpenoids	+
9	Steroids	+
10	Saponins	-
11	Tannins	+
12	Quinones	-
13	Resins	-

Inorganic mineral analysis

The mineral composition of *P. hornemannii* was listed in Table. The presence of different minerals and their quantitative amount is also represented.

Table 4: Inorganic analysis of methanolic extract *P. hornemannii*

S.NO	Inorganic compounds	Amount of presence
1	Cadmium	0.18 ± 0.01
2	Calcium	3.89 ± 1.15
3	Cobalt	0.74 ± 0.11
4	Copper	0.26 ± 0.07
5	Iron	91.7 ± 3.08
6	Lead	ND
7	Magnesium	16.8 ± 1.83
8	Nickel	ND
9	Zinc	3.8 ± 0.04

10	Manganese	2.5 ± 2.49
11	Potassium	752.9 ± 5.72

Fluorescence analysis

The characteristic fluorescent properties or colour emitted by the powdered sample *P. hornemannii* before and after treating with various reagents were catalogued. The powdered sample under a visible microscope was appeared to be an orange reddish colour and fluorescent greenish dark red colour under ultraviolet radiation. After treating with various reagent in the powder sample showed different colour listed in table 5

Table 5 shows the fluorescent analysis of red seaweed *P. hornemannii*

Treatment	**Visible light**	**UV light 366nm**
Dry powder	Reddish orange	Greenish fluorescent red
Powder + 50% H_2SO_4	Blackish red	Greenish black
Powder + Conc H_2SO_4	Dark red	Greenish dark red
Powder + 50% HCL	Blackish red	Greenish black with an orange shade
Powder + Conc HCL	Dark red	Greenish black with an orange shade
Powder + 50% HNO_3	Blackish red	Greenish black with an orange shade
Powder + Conc HNO_3	Dark red	Greenish red with an orange shade
Powder + 1 N HCl	Blackish red	Greenish black
Powder + 5% KOH	Dark Reddish black shade	Black
Powder + 10% NaOH	Dark reddish orange	Greenish red

GC-MS

The GC-MS chromatograms showed a legion of metabolites in methanolic extract of *Portieria hornemannii.* Among the N number of metabolites the predominant metabolites was listed in Table (6) with their retention time (RT), Name of the Compound, Molecular Formula, Molecular weight (MW), and Peak area %. The figures (2, 3, 4 & 5) shows the presence of predominant metabolites with its retention indices and figure 6 represents the

chromatogram of metabolites present in the methanol extract of *Portieria hornemannii* provides the result of different peaks determining the presence of seven different compounds.

Table 6: Composition of *Portieria hornemannii* methanolic extract was investigated by GC-MS chromatography

S.No	Retention Time	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.86	2-Pentadecanone,6, 10, 14, Trimethyl,	$C_{18}H_{36}O$	268	84.00
2	18.08	Oxirane [(Hexadecyloxy) Methyl]-	$C_{19}H_{38}O_2$	298	33.18
3	18.45	Heptacosanoic acid, Methyl ester	$C_{28}H_{56}O_2$	424	38.01
4	19.09	Heptacosanoic acid, 25-Methyl- Methyl ester	$C_{29}H_{58}O_2$	438	42.86
5	19.89	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	99.97
6	21.69	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	47.03
7	27.92	Lauroyl peroxide	$C_{24}H_{46}O_2$	398	67.91

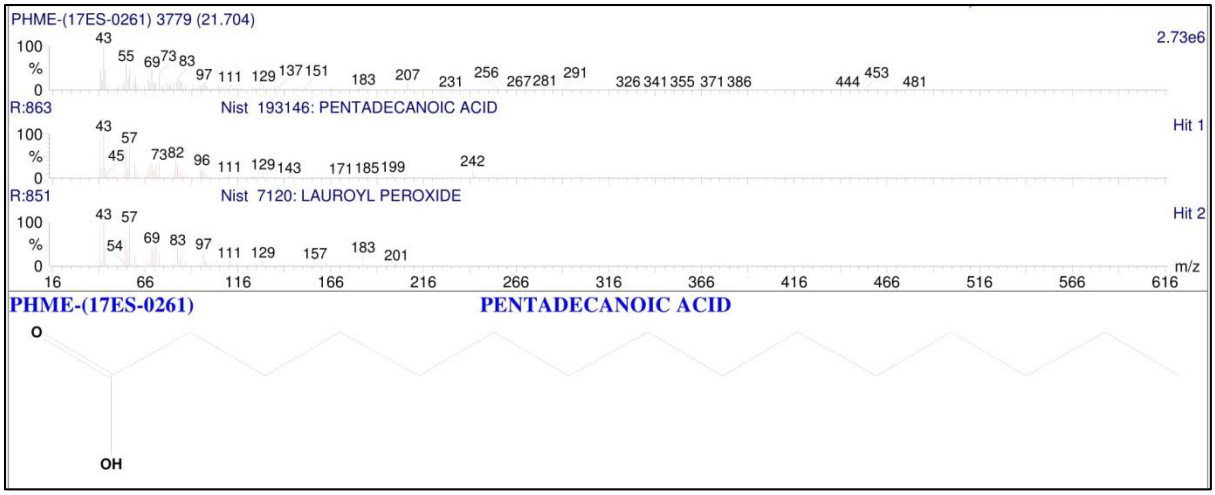

Figure 2: GC-MS shows the retention indices of compound Pentadecanoic acid and Lauroyl peroxide in methanolic extract of *Portieria hornemannii*

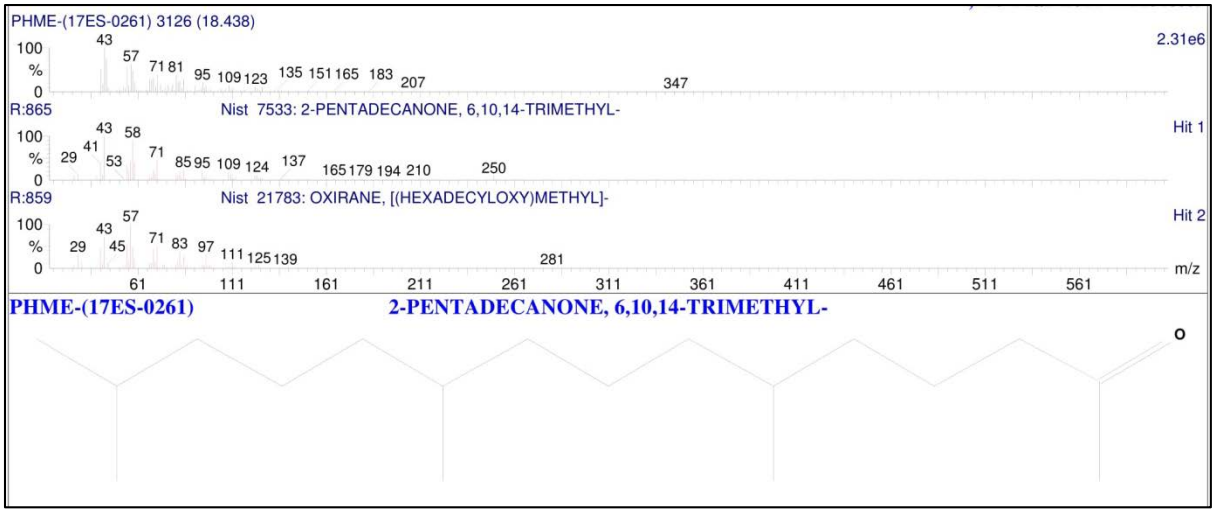

Figure 3: GC-MS shows the retention indices of compound 2-Pentadecanone, 6, 10, 14, Trimethyl and Oxirane [(Hexadecyloxy) Methyl]- in methanolic extract of *Portieria hornemannii*

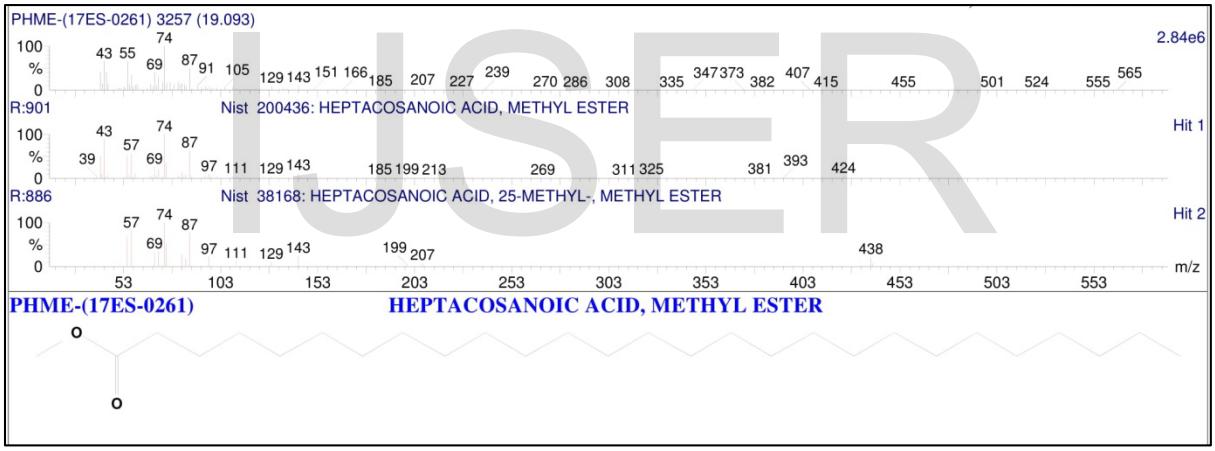

Figure 4: GC-MS shows the retention indices of compound Heptacosanoic acid, Methyl ester and Hepatacosanic acid, 25-Methyl-, Methyl Ester in methanolic extract of *Portieria hornemannii*

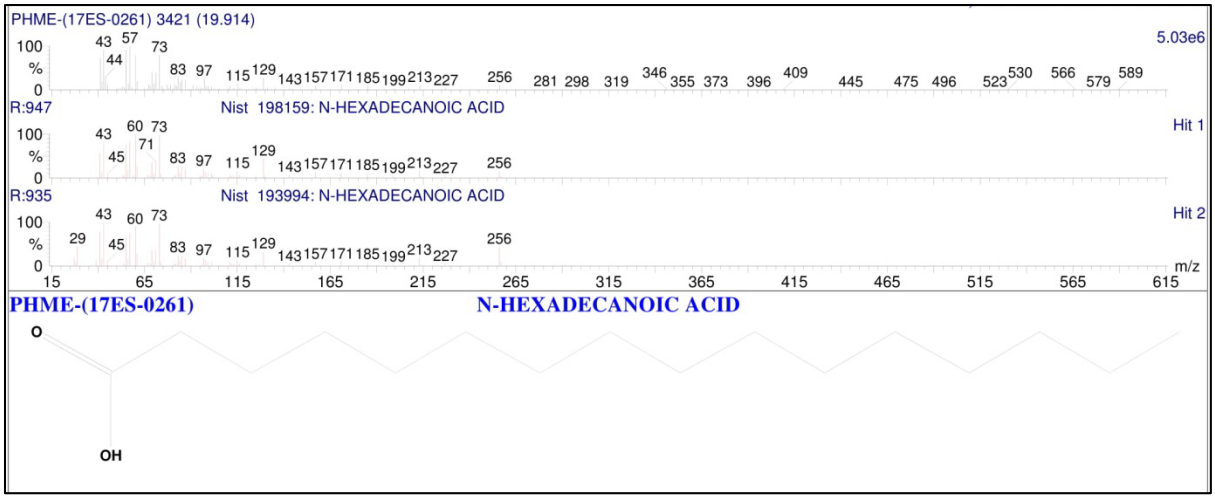

Figure 5: GC-MS shows the retention indices of compound N-Hexadecanoic acid in methanolic extract of *Portieria hornemannii*

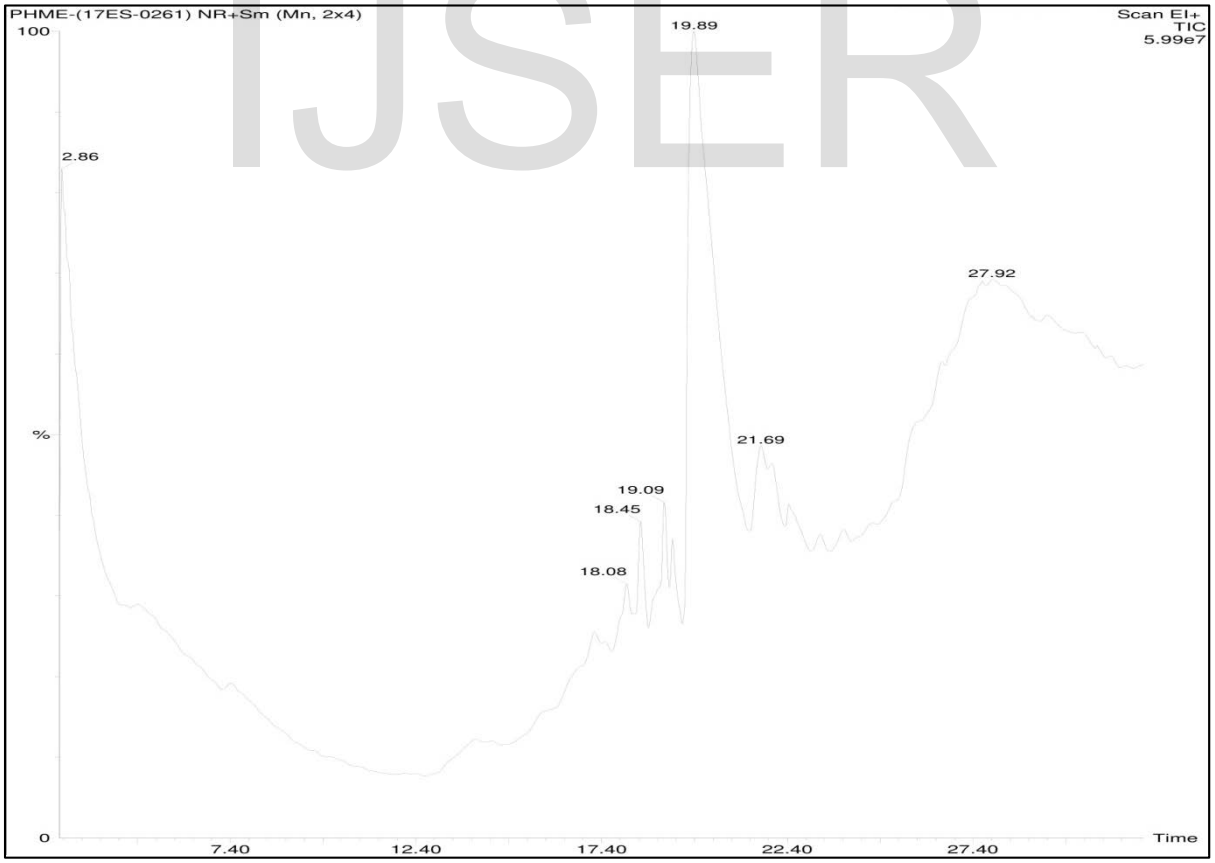

Figure 6: GC-MS chromatogram of *Portieria hornemannii* methanolic extract

DISCUSSION

Plants play a major role in the pharmaceutical field. It was well known in recent years that seaweeds are considered to be rich in the source when compared to the terrestrial plants. In order to use these plants or seaweed in an effective way, standardization is an important tool to produce herbal drugs. The identity, purity, safety and quality are considered to be predominant criteria to standardize a herbal drug. To produce a successful drug, various macroscopic, physicochemical analyses, phytochemical analysis, inorganic mineral analyses, and fluorescence analysis were done. The quantitative determinations of some pharmacognostical parameters are useful for setting standards for crude drugs. Pharmacognostical parameters for easy identification like leaf constituents, microscopy and physicochemical analyses are few of the basic protocol for standardization of herbals (Dinakaran *et al.*, 2011). The preliminary phytochemical screening will reveal the nature of the drug and physicochemical analysis will be helpful in the identification and authentication of the plant material (Kumar *et al.*, 2011).

Both macroscopic and microscopic studies provide physical and morphological information for the identification and authentication of *P. hornemannii*. The physicochemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. It is an important valuable source of information that will provide appropriate standards to establish the quality of seaweed *P. hornemannii* for its advanced research or application (Subramanian Sampathkumar and Ramakrishnan, 2011).

The phytochemical screening revealed the presence of a list of phytochemicals in the *P. hornemannii*. Several reports justified the presence of phenols and flavonoids in seaweeds and have been reported to exhibit several biological activities including antioxidant activity (Chandini *et al.*, 2008). The antimicrobial properties of Alkaloids are commonly found

against both Gram-positive and Gram-negative bacteria (Cowan, 1999). Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects (Xu *et al.*, 2000). The presence of these secondary metabolites brings an understanding that the seaweed might be of medicinal importance and can be used as anti-microbial, anti-parasitic, anti-inflammatory, anti-oxidant agents.

The mineral composition of the *P. hornemannii* exhibited high amounts of potassium, magnesium and iron, but low concentrations of cadmium, copper and cobalt, similar to values reported by Krishnaiah *et al.* (2008), but relatively higher than those reported by Rupérez (2002). The level of potassium is very high (752.9 mg/100g) when compared to 11 minerals quantified but this was comparably lower than those reported in seaweeds examined by Matanjun *et al.* (2009) (8371.2–13155.2 mg/100 g). Magnesium content (16.8 mg/100g) in the red seaweeds are little lower than those determined by Manivannan *et al.* (2008b) in U. lactuca (17.5 mg/100 g) and higher than H. valentiae (4.0 mg/100 g), and compatible to those estimated by Matanjun *et al.* (2009). The concentration of manganese (2.5 mg/100g) is comparably low wherein H. valentiae it is 3.7 mg/100 g. the seaweed contains a significant amount of iron when compared to the copper in this study were higher than those reported by McDermid and Stuercke (2003). Seaweeds have a tendency to absorb minerals selectively from the surrounding seawater and accumulate them in their thalli (Azmat *et al.*, 2006) hence; their mineral composition and concentration depend on the species and location.

The fluorescence analysis is an ample reactive and enables the unambiguous and accurate determination over the concentration range of crude without several time-consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al.*, 2006). The characteristic fluorescent properties or colours of the *P. hornemannii* recorded through this study could be used as a standard in the recognition and authentication of the red seaweed *P. hornemannii* in its crude form.

CONCLUSION

Marine algae are proficient source of unsaponifiable nontoxic steroids and other secondary metabolites. The present investigation brings out adequate data on the phytochemical constituents present in the seaweed. The presence of phytochemical mixtures makes the seaweeds useful for treating different affliction and has the potential of presuming useful drugs for human use. These chemical constituents possess antibacterial, antiviral, antifungal, anticoagulant, antitumor and anti-inflammatory activities. The red seaweed species is an emerging one in pharmaceutical field for which evidence of pharmacognosy is lacking, so in the present study, Pharmacognostic standards have been exposed to highlight biological importance in the Pharmaceutical field.

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