Identification of Novel Metabolites along with the Assessment of Metal Toxicity and Its Implication in Marine Red alga Hypnea musciformis

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Abstract
Marine algae are known to contain a wide variety of bioactive compounds, biomaterials derived from marine algae have commercial applications in pharmaceutical, medical, cosmetic, nutraceutical, food and agricultural industries. Marine algae are found to contain high amount of nutrients, vitamins (A, B, C, D & E), minerals (Ca, P, Na & K), antioxidants and dietary fibers, that’s why they are used as food, fodder and other commercial purposes throughout the world. Seaweeds have been used as an important source of food because of their biochemical composition. They are also very rich in novel compounds and can be explored for the development of drugs to combat deadly diseases like cancer, diabetes etc. The present study indicates the presence of bioactive compounds by using HPLC and FTIR in marine algae Hypnea musciformis. It includes polysaccharides, terpenes, alkenes, sterols etc. These results indicated that seaweeds H. musciformis could be potential sources of development novel bioactive compounds. Similarly the heavy metals analyzed were found in less concentration and does not have any effects. It was (Cd, Fe, Cu, Ca, Cr, Pb, Ni, Co, Mn, Zn) metals were estimated using atomic absorption spectroscopy. Seaweeds can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of seaweed-based products. In the future, these marine algae-derived materials/compounds will be used more often in pre-clinical studies for drug discovery.

Keywords
H. musciformis; Heavy metals; Bioactive compounds; HPLC; FTIR

Introduction
The world’s oceans, covering more than 70% of the earth’s surface, represent an enormous resource for the discovery of potential therapeutic agents. During the last decades, numerous novel compounds have been found from marine organisms with interesting pharmaceutical activities. Therefore, marine organisms are believed to be a potential source to provide not only novel biologically active substances for the development of pharmaceuticals (Burja et al., 2001). Seaweeds are classified as Green algae (Chlorophyta), Brown algae (Phaeophyta), Red algae (Rhodophyta) and some filamentous Blue-green algae (Cyanobacteria). Most of the seaweeds are red (6000species) and the rest known are brown (2000 species) or green (1200 species) (Kohen and Nyska, 2002). Marine algae are rich in dietary fiber, minerals lipids, proteins, omega-3 fatty acids, essential amino acids, polysaccharides, and vitamins A, B, C, and E. Studies on the bioactivities of marine algae have revealed numerous health-promoting effects, including anti-oxidative, anti-inflammatory, antimicrobial, and anti-cancer effects (Tabarsa et al., 2012). The use of marine natural products able to inhibit bacteria development offers a rich pharmacological potential. Numerous reports show that macroalgae present a broad range of biological activities such as antibacterial antifungal antiviral and anti-inflammatory (Etahiri et al., 2003) In contrast to the brown and green algae, the red algae are more known to produce halogenated metabolites, particularly bromine and iodine. To date, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals compounds (Lima Filho et al., 2002).

Distinctive structure of viruses and their complicated lifecycle have made the discovery of definite treatments against antiviral infections extremely demanding. Despite comprehensive Studies for suitable vaccines and
treatments against viral infections over the past half of a century, still several infections, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), and dengue virus (DENV), afflict a substantial proportion of the world populations in all generations (Lazarus et al., 2014). The unique living environment has gifted the marine world an assorted collection of algae from microorganisms to giant seaweeds. Various types of algae from microscopic diatoms to unicellular organisms and seaweeds reaching 30m in length have stimulated significant economic interest’s agar, fertilizer, food, source of iodine, and potash (Yasuhara and Lu, 2010). However, algal based bio sorbents can be modified by a treatment of ferric chloride (FeCl3) followed by paralysis to yield a Fe-treated biochar with a high bio sorption capacity for Serve in solution (Roberts et al., 2014). One potentially abundant source of biomass for bio sorption applications is the use of the waste biomass produced in the commercial extraction of agar from cultivated red seaweeds. The main genus of algae grown for the extraction of agar is Gracilaria. Commercial cultivation of Gracilaria is increasing rapidly worldwide, particularly in Indonesia where more than 500,000 tonnes are produced annually (Siben and Calderini, 2012). Many substances obtained from marine algae such as alginate, carrageenan and agar as phycocolloids have been used for decades in medicine and pharmacy fields. They showed bacteriostatic and bactericidal activity. Among the natural products substances like amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acids etc are seen in marine algae (Kidgel et al., 2014). Anti-inflammatory properties have been reported for only two species of red algae G. verrucosa and G. textorii The anti-inflammatory effects of a methanol extract of Neorhodomela aculeate in neurological diseases included inhibiting cellular reactive oxygen species (ROS) generation, H2O2 induced lipid per oxidation, and inducible nitric oxide synthase (Lee et al., 2011).

Macro algae in the Red Sea are adapted to living in an environment with specific demands. The temperature is often above 35°C, and the specific nutrients available define their cellular compositions. Macro algae express highly bioactive compounds that may be exploitable as antimicrobial agents to support both human and animal health. The isolation and identification of new compounds with potential health or pharmaceutical and medicinal activities have attracted intensive research efforts (Kajiwara et al., 2007). Several studies have shown the antibacterial effects of macroalgal and plant extracts, including those from the algae Padina tetrostomatica Syringodium isoetifolium, Haligra sp. Gelidiella acerosa, Laminaria digitata, L. saccharina, Himanthalia elongate, Palmaria palmate, Chondrus crispus and Enteromorpha spirulina, and plants Dortenta picta and Bridelia micrantha against several pathogenic bacteria (Gupta and Abu, 2011). The ability to produce bioactive substances may be noticed not only as a defense mechanism but also as a good source of new bioactive compounds from a pharmaceutical point of view. Recently bioactive allele chemical compounds from Oscillatoria species (Egyptian isolates). Many unique compounds of fresh water origin with various biological activities have been isolated and some of them are under investigation to develop new pharmaceuticals (Elsie and Dhanarajan, 2010).

Materials and Methods
Collection of marine alga
Marine alga samples are collected from the coastal areas of Tuticorin at the south east coast if India. The taxonomic identification of the species was identified as Red algae Hypnea musciformis.

Extract preparation
Collected samples were washed with tap water to remove epiphytes and other marine organisms and then washed with distilled water. Samples were shade dried and powdered. The extracts were prepared using the solvent methanol.

FTIR Analysis
The FT-IR analysis of sample was carried for marine algal extracts. All the IR spectra were recorded at room temperature (26°C or 1°C) in the mid infrared range (40000-400 cm⁻¹) using FT-IR – spectrum RX I, Fourier Transform Infrared Spectrometer (PerkinElmer, USA). Typically, 20 scans were signal-averaged for a single spectrum. Each spectrum was displayed in terms of absorbance as calculated from the reflectance- absorbance spectrum using the Hyper-IR software.
HPLC analysis
The extracts of each specimen were prepared quantified by HPLC. From the 1.5 ml volume of each crude extract saved for HPLC quantification, 200 µl were transferred to a vial and the solvent removed by Speed-Vac vacuum concentration. The obtained residue was dissolved in 500 µl acetonitrile:water 1:1 + 0.5% trifluoroacetic acid and 10 µl injected by auto-sampling into a HPLC.

Determination of Heavy Metals
The extracts of marine Red algae *Hypnea musciformis* were then poured into auto analyzer cups and concentration of heavy metals (Cd, Fe, Cu, Ca, Cr, Pb, Ni, Co, Mn, Zn) was determined using Atomic Absorption Spectrophotometer (AAS). The values of the heavy metal concentrations in the extracts was calculated based on ppm.

RESULTS
The marine Red alga samples were collected from Tuticorin coast (Fig 1). The taxonomic position of the algae was analyzed. Fig 2 explains the detection of marine bioactive compounds from the algae *Hypnea Musciformis* using FTIR analysis. Fucoidans are a class of sulfated fucose-rich polysaccharides are seen. Then carotenoid, fucoxanthin triterpene glycosides are also observed. The methanol extracts of *Hypnea musciformis* were then subjected to HPLC and analysis for the detection of the synthesized natural compounds. Fig 1 shows the detection of marine derived natural products from the algae *Hypnea musciformis* like β-carotenoid and vitamin E. Then Macroalgae-derived phytosterols can be divided into four groups, 4-desmethyl-Δ5-sterols, 4-desmetyl-Δ7-sterols, 4-methyl sterols and dihydroxylated sterols. HPLC analysis showed that the most abundant phenolic compound was epicatechin (i.e., gallic acid, 4-hydroxybenzoic acid, catechin hydrate, epicatechin, catechin gallate, epicatechin gallate, epigallocatechin, epigallocatechin gallate, pyrocatechol). (Figure 3 and Table 1 show the presence pf heavy metals (Cd, Fe, Cu, Ca, Cr, Pb, Ni, Co, Mn, Zn) in the algae.

| S.NO | Element | Wavelength | Fuel gas flow rate (L/min) | Flame type (L/min) | Concentration of metals in ppm |
|------|---------|------------|---------------------------|-------------------|-------------------------------|
| 1    | Cadmium | 228nm      | 1.8                       | 15                | 0.0490                        |
| 2    | Iron    | 248nm      | 2.2                       | 15                | 0.2768                        |
| 3    | Copper  | 324nm      | 2                         | 15                | 0.8799                        |
| 4    | Calcium | 422nm      | 2                         | 15                | 30.9366                       |
| 5    | Chromium| 357nm      | 2.8                       | 15                | 0.5322                        |
| 6    | Lead    | 283nm      | 2                         | 15                | 0.3643                        |
| 7    | Nickel  | 232nm      | 2                         | 15                | 0.0242                        |
| 8    | Cobalt  | 240nm      | 1.6                       | 15                | 0.0922                        |
| 9    | Manganese| 279nm     | 2                         | 15                | 2.5210                        |
| 10   | Zinc    | 213nm      | 2                         | 15                | 1.6194                        |
DISCUSSION
Natural products play a valuable role discovery. Therefore, the search for novel algal natural compounds should open promising areas of pharmaceutical study. Many reports of biologically active compounds extracted from algae have been published, and confirm their ability to produce metabolites with highly complex and remarkably diverse pharmacological and biological properties. 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 algae Ulva fasciata, Caulerpa scalpelliformis, Halimeda macroloba, Enteromorpha compressa, Caulerpa corynephora and Ulva lactuca revealed the presence of a good number of secondary metabolites (De Almeida et al., 2011).

Figure 2 FTIR analysis of marine Red alga Hypnea musciformis

In the present study the red algae contains secondary metabolites like alkenes, sterols, terpenes etc. Marine algae have been traditionally used as food and medicine. It is because they contain the essential amino acids and polyunsaturated fatty acids, necessary vitamins and minerals, and larger amounts of dietary fibers. Other reports indicated that marine algal polysaccharides and proteinaceous substances have valuable functions in immune modulation and as well as in lowering blood pressure, cholesterol, and glucose level (Thomas and Kim, 2011). Seaweeds have afforded to date the highest number of compounds within a single group of marine organisms. Among the bioactive red algal metabolites, dehydrothyrsiferol a triterpenoid polyether isolated from a Canary island collection of Laurencia viridis, has exhibited promising cytotoxic activity. Therefore, its cytotoxic effect on human estrogen receptor ER+ and ER- breast cancer cell lines was studied in a preclinical pharmacological evaluation [17]. Three different fucoidan fractions were isolated and purified from the brown alga, Sargassum meclurei. The SmF1 and SmF2 fucoidans are sulfated heteropolysaccharides that contain fucose, galactose,
mannose, xylose and glucose and SmF3 fucoidan is highly sulfated (35%) galactofucan. All fucoidans fractions were less cytotoxic and displayed colony formation inhibition in colon cancer DLD-1 cells (Kim et al., 2010). The aqueous extract of algae *Gracilaria corticata* from the Persian gulf showed antitumoral activity on Jurkat and molt 4 human lymphoblastic leukemic cell lines (Yanti et al., 2015). Phaeophyceae or brown algae revealed marked antitumor activity, and the sulfated polysaccharide of fucoidan and carotenoid of fucoxanthin were found to be the most important active metabolites of brown algae as potential chemotherapeutic or chemopreventive agents (Cornish and Garbary, 2010). Green alga *Bryopsis sp.* was the source of the cyclic depsipeptides Kahalalides P and Kahalalides Q with moderate inhibition of the HL-60 cell lines. The cyclic depsipeptide Kahalalide F was originally isolated from both mollusc *Elysia rufescenes* and from the dietary source, the green alga *Bryopsis sp.* It was introduced into Phase I trials by Pharma Mar as a lead compound against prostate cancer (Chen et al., 2013). Maximum levels of calcium is found in the red algae *Hypnea musciformis*. Traces of nickel and cobalt are seen. Manganese and Zinc are found little high levels.

![HPLC analysis of *Hypnea musciformis*](image)

**Figure 3** HPLC analysis of *Hypnea musciformis*

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**Conflict of Interest:** There are no Conflicts of interest.
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