Evaluation of Natural Products from Marine Algae gelidiella indica and Acanthophora flagelliformis

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Abstract: The methanol extracts of marine algae collected from the coast of Kilakarai such as Gelidiella indica and Acanthophora flagelliformis showed the presence of marine derived natural compounds. The Phytochemical analysis showed the presence of compounds like Alkaloids, saponin, carbohydrate, phenol, flavonoids, phystosterols, terpenoids, cardiac glycosides. The HPLC analysis using the crude extracts of algae was done to evaluate and identify the presence of natural compounds. It emphasizes the role of marine algae as an important source of leads for drug discovery due to the presence of bioactive compounds. It is also found out that the marine derived algae compounds have medicinal effects and could be used in drug development.

Key words: Gelidiella indica • Acanthophora flagelliformis • HPLC

INTRODUCTION

Marine algae are one of the largest producers of biomass in the marine environments. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine macro and microalgae and have antibacterial, antialgal, antimacrofouling and antifungal properties [1]. Commercially available varieties of marine macroalgae are commonly referred to as seaweeds. Macro algae can be classified as red algae (rhodophyta), brown algae (phaeophyta) or green algae (chlorophyta) depending on their nutrient and chemical composition [2]. Seaweeds serve as an important source of bioactive natural substances. They have some of the valuable medicinal value components such as antibiotics, antioxidant, anticoagulants, anti-ulcer and antibacterial activity against human pathogens. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas [3]. The cytotoxic activities were seen in Sargassaceae from Desmarestia ligulata and Dictyota dichotoma. Many substances obtained from marine algae such as alginate, carragenean and agar as phycocollids have been used for decades in medicine and pharmacy fields. They showed bacteriostatic and bactericidal activity [4]. Evaluation of phycochemical and pharmacological studies on algae indicates the presence of various biochemical compounds such as the amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides [5]. Reactive oxygen species (free radicals) are highly reactive chemical molecules that are formed as by-products of normal metabolism and whose levels increase dramatically due to various environmental stresses or damage. They finally cause great damage to the cells by interacting with vital cellular components like DNA or the cell membrane. Antioxidants play an important role in preventing cellular damage—a major feature of cancer, ageing and various other diseases-by neutralizing this free radicals [6]. Seaweeds have significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial activities. Many metabolites isolated from marine algae have been shown to possess bioactive efforts. Brown seaweeds have been used in traditional medicine. Sargassum graminifolium, is brown seaweed, commonly consumed as seafood and as medical resource for its antiinflammatory effects [7]. Marine algae are valuable sources of structurally diverse bioactive compounds. Sulfated polysaccharides are widespread in marine algae,
especially brown seaweeds. Sulfated polysaccharides show various biological activities, including anticoagulant, antioxidant, antiviral, anticancer and immunomodulating activities [8]. The detected antioxidant compounds in algae from chlorophyta and rhodophyta have potential anti-aging, dietary, anti-inflammatory, antibacterial, antifungal, cytotoxic, anti-malarial, anti-proliferative and anticancer properties [9]. Natural products and some of their derivatives have been reported to exhibit remarkable growth inhibitory activity towards M. tuberculosis and some of them have been selected as prototype molecules for the development of new antitubercular agents. The secondary metabolites isolated from algae, which were grouped according to their chemical type as terpenes (sesquiterpenes, diterpenes, sesterterpenes, triterpenes), steroids (sterols), alkaloids (indole, quinoline, pyridoacridone and manzamine alkaloids, etc [10]. Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Brazilian red algae have been found to have phenolic substances. Oxidative stress is an important factor in the genesis of pathology, from cancer to cardiovascular and degenerative disease. Marine algae are the excellent source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals [11]. In this present study an attempt has been made to analyze the marine derived natural products from marine algae collected from the region of Kilakarai in Ramanathapuram district in Kilakarai at the south east coast if India. The taxonomic position of the species was identified as Gelidiella indica and Acanthophora flagelliformis.

**Preparation of Extracts:** The algae after drying were weighed and then chopped. Algae extracts were prepared by using direct extraction method. The samples were weighed and dissolved in methanol. It was kept for 24 hrs at room temperature and mixed at regular intervals. After 24hrs the dissolved samples were filtered using Whatman filter paper and stored for further use.

**Phytochemical Analysis:**

A) Detection of Alkaloids: Preparation of filtrate solvent free extract (50 mg) was stirred with 2 ml of dilute hydrochloric acid and filtered. To 1 ml of filtrate a drop of Mayer’s reagent was added by the side of tube and then observed for a white creamy precipitate;

B) Detection for Saponins: Two grams of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

C) Detection of Carbohydrates: To 0.5 ml of filtrate 0.5 ml of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 min and after that observed for characteristic red colored precipitate formation.

D) Detection of Phenolic Compounds: The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral ferric chloride solution was added and observed for a dark green coloration.
E) Detection of Flavonoids: To 5 ml of dilute ammonia solution a portion of the aqueous filtrate of each algal extract followed by addition of concentrated sulfuric acid and then observed for a yellow coloration. The yellow coloration disappears on standing.

F) Detection of Phytosterols: The extract (50 mg) was dissolved in 2 ml of acetic anhydride. To this 1 or 2 drops of concentrated sulphuric acid was added slowly along the sides of the tube and observed for an array of colour. 

G) Detection of Terpenoids: Salkowski test: 5 ml of each extract was mixed in 2 ml of chloroform and concentrated sulfuric acid was added to form a layer and then it was observed for reddish brown coloration of the interface.

H) Test for Cardiac Glycosides: Keller-Killani test: 5 ml of each extract was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. It was added with 1 ml of concentrated sulfuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout thin layer.

HPLC Analysis: The crude 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.

RESULTS AND DISCUSSION

The marine algae samples were collected from Kilakarai area in Ramanathapuram district. The methanol extracts were prepared from the algae a) Gelidiella indica b) Acanthophora flagelliformis. The phytochemical analysis were done to detect the presence of Alkaloids, saponin, carbohydrate, phenol, flavonoids, phyosterol, terpenoids, cardiac glycosides. In Gelidiella indica all the active compounds are seen whereas in Acanthophora flagelliformis saponins and phyosterols are absent (Table 1). Based on the HPLC analysis Fig. (1) explains the detection of marine derived natural products from the algae Gelidiella indica such as galacto glycosphingolipids, glycosides, sphingolipids and cyclic peptides based on the retention time. Fig (2) reveals the detection of marine derived natural products from the algae Acanthophora flagelliformis, such as sphyngolipids, sesterpenoids, sphingolipids, phenolic compounds and flavones glycosides based on the retention time. Algae are group of marine plants and they are not only primary and major producers of organic matter in the sea but they also exert profound effects on the density and distribution of their inhabitants of the marine environment. Algae contain rich and largely entrapped sources of a vast assortment of biologically active substances [12]. Hence it is assumed that the marine algae collected from the coast of Kilakarai have lot of chemical compounds and it may have various in vitro effects.

Centeno and Balantine [13] reported that the presence of chemical compounds have already isolated from algae are provide valuable ideas for the development of new drugs against different diseases. Many marine algae produce antibiotic substances capable of inhibiting bacteria, viruses, fungi and other epibionts. It appears that the antibiotic characteristic is dependent on many factors, including the particular alga, the microorganisms, the season and the growth conditions. The antibacterial activity of marine algae is generally assayed using extracts in various organic solvents, e.g., acetone, methanol, toluene Diethylether, ethanol and chloroform-methanol. Several extractable compounds, such as cyclic polysulfides and hologenated compounds are toxic to microorganisms and therefore responsible for the antibiotic activity of some marine algae [14]. Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry Croke et al. [15]. As a consequence of an increasing demand for biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in marine organisms, especially algae Devery et al. [16]. Screening of seaweeds for antimicrobial activity and bioactive constituents is quite imperative. It is well known that many drugs can be prepared from marine source which could profitably be used in pharmaceutical industries. There is a growing demand and need for new marine derived drugs to control many bacterial and fungal diseases Mori et al. [17]. From the present work it is concluded that the marine algae species such as the a) Gelidiella indica b) Acanthophora flagelliformis have lot of natural compounds, hence it can be used as for the development of drugs.
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<th>Tests</th>
<th>Gelidiella indica</th>
<th>Acanthophora flagelliformis</th>
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<td>Alkaloids</td>
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Table 1: Phytochemical analysis of marine algae

Fig. 1: HPLC analysis of marine algae Gelidiella indica

Fig. 2: HPLC analysis of marine algae Acanthophora flagelliformis

REFERENCES


