CHEMICAL INVESTIGATION OF BROWN SEAWEED – *Padina pavonica*

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DECLARATION

I certify that this dissertation, which was practically implemented by me at the Institute of Fundamental Studies and the Faculty of Applied Sciences under the supervision of Prof.H.R.W.Dharmaratne and Prof.D.B.M.Wickramaratne, does not incorporate with out acknowledgment of any material previously submitted for a degree or diploma in any other university and to the best of my knowledge and belief this does not contain any material previously published in writing or orally communicated by another person where due reference is made in the text.

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Affectionately
Dedicated
To My Parents
&
To All My Teachers

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ABSTRACT

In recent years research on the chemistry of seaweeds (or more generally marine algae) has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties. Marine algae (Seaweeds) are known to produce an incredible diversity of secondary metabolites some of which show potent anticancer, antibiotics, antiviral antimicrobial, anti-inflammatory, anthelmintic, antifungal, antitumor and insecticidal activities. Considerable amount of work have been done on seaweeds around the world. However, the biomedical potential of the rich Sri Lankan seaweeds remains largely unexplored. Due to this reason Natural product program of the Institute of Fundamental studies initiated a project on chemistry and biological activity studies of Sri Lankan seaweeds.

Padina pavonica, brown algae was collected from the coastline of Arugambay at Ampara District in the Eastern Province in Sri Lanka. Plant material was cleaned, washed with fresh water, air dried, powdered and extracted with MeOH using a sonicator. Dried MeOH extract was successively fractionated with Hexane, Chloroform and Ethyl acetate.

After that the dried Hexane fraction was subjected to column chromatography with normal Silica and C-18, followed by Preparative Thin Layer Chromatography (PTLC) to gave three pure compounds but the amount of these compounds were small and needed high sensitive instruments to analyze them.

As I was not in a position obtain the H NMR of isolated compounds, for familiarization of spectroscopic techniques, spectral data of a compound isolated from green seaweed, *Ulva lactuca* was analyzed (^{1}H and ^{13}C NMR, HMQC and HMBC Spectrums) and its' structure was proposed as 9-octadecenoic acid (Oleic Acid).

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LIST OF ABRIVIATIONS

^0C	-	Degrees of Celsius
atm	-	Atmospheric pressure
mg	-	Milligrams
g	-	Grams
Kg	-	Kilo gram
mm	-	Millimeter
cm	-	centimeter
m	-	Meter
Km	-	Kilometer
Min.	-	Minutes
^{13}C NMR	-	Carbon-13 Nuclear Magnetic Resonance
^1H NMR	-	Proton nuclear Magnetic resonance
HMBC	-	Heteronuclear Multiple Bond Coherence
HMQC	-	Heteronuclear Multiple Quantum Coherence
DEPT	-	Distortionless Enhancement by Polarization Transfer
MeOH	-	Methanol
EtOAc	-	Ethyl Acetate
$CDCl_3$	-	Deuterio chloroform
TLC	-	Thin Layer Chromatography
PTLC	-	Preparative thin Layer Chromatography
R_f	-	Retention Factor
Hz	-	Hertz
MHz	-	Mega Hertz
ppm	-	Parts per million
m	-	Multiplet
t	-	Triplet
δ	-	Chemical shift position
I	-	Spin quantum number
J	-	Coupling constant

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CHAPTER 1
INTRODUCTION

1.1 Introduction

In recent years research on the chemistry of seaweeds (or more generally marine alga) has experienced a tremendous increase due to the need for compounds possessing bioactivities of possible pharmaceutical applications or other potential economic properties. To this end, a variety of species have been assayed for their activity and a number of biodynamic molecules, often with toxic properties and unique structural features, have been isolated. Since marine organisms live in a significantly different environment from those of terrestrial organisms, it is reasonable to suppose that their secondary metabolites will differ considerably. After more than 25 years of fruitful research, marine natural product chemistry must now be considered to be approaching maturity. If the novelty and complexity of compounds discovered from marine sources were the only criteria, then the success of research in this area would be assured, for there are many marine natural products that have no counterparts in the terrestrial world. (Shaik Ali et al., 1999)

The origination of all of living organisms on the earth occurs in the ocean, which is cover more than 70% of the earth' surface and are an indispensable source of protein for human nutrition. The ocean is also the source of structurally unique natural products that are mainly accumulated in living organisms. Several of these compounds show pharmacological activities and are helpful for the invention and discovery of bioactive compounds, primarily for deadly diseases like cancer, acquired immuno- deficiency syndrome (AIDS), arthritis, etc., while other compounds have been developed as analgesics or to treat inflammation, etc. The lifesaving drugs are mainly found abundantly in algae and modern technologies have opened vast areas of research for the extraction of biomedical compounds from oceans and seas.

The marine environment may contain over 80% of world's plant and animal species and only 10% of over 25,000 plants have been investigated for biological activity. The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites many of which are endowed with pharmacodynamic properties. Even though due to the ethno-medical history and difficulty in the collection of marine algae, research into the use of marine natural products as pharmaceutical agents is still in its

infancy. But with the development of new diving techniques, remote operated machines, etc., it is possible to collect marine samples and during the past decade, over 5000 novel compounds have been isolated from shallow waters (Jha and Zi-rong, 2004).
well over 14,000 different natural products from marine organisms have been described hundreds of patents describing new bioactive marine natural products have been filed and approximately 10–15 different marine natural products are currently in clinical trials mostly in the areas of cancer, pain or inflammatory diseases (Peter et al., 2003). Seaweeds are abundant in the intertidal zones and in clear tropical waters and they have received comparatively less bioassay attention (Jha and Zi-rong, 2004).

Sri Lanka is an island (7 00 N, 81 00 E), lies in the Indian Ocean, to the southwest of the Bay of Bengal and to the southeast of the Arabian Sea. It consist with 65610 Km^2 total areas, including 64740 Km^2 as land and 870 Km^2 as inland water bodies with the 1340 Km coastline (The world factbook, 2007).

The large amount of work have been done on seaweeds around the world, but the biomedical potential of the rich Sri Lankan seaweeds remains largely unexplored. Marine algae have been found abundant particularly along the Northern and Southern coastal regions of Sri Lanka. The algal vegetation along the coasts shows distinct floristic associations and seasonal variations. In a floristic survey in 1961, the presence of 315 species of algae distributed among the orders Chlorophyta, Phaeophyta and Rhodophyta has been recorded from the coastal areas in Sri Lanka (Bandara et al., 1988). So it is very important and opportune to study about chemistry and biological activity of seaweeds around Sri Lanka.

Due to the increasing demand of therapeutic drugs from natural products, aggrandize interests of scientists and pharmaceutical companies on marine organisms, especially on seaweeds. But studying programs on three classes of marine algae is not uniform. Most of the studies done on green algae, but chemically brown algae are most important. But the studies on Brown algae are scanty.

1.2 Objectives

1.2.1 Overall Objective

Isolating of natural products from brown marine algae –*Padina pavonica* with the hope of finding compounds with biological activities.

1.2.2 Specific objectives

- Identify the compounds by elucidation the structures of isolated compounds
- Familiarization with spectroscopic techniques and other analytical techniques

CHAPTER 2
LITERATURE REVIEW

2.1 Marine Organisms

Marine life is concerned with the plants, animals and other organisms that live in the ocean. Given that in biology many phyla, families and genera have some species that live in the sea and others that live on land, marine biology classifies species based on the environment rather than on taxonomy. For this reason marine life encompasses not only organisms that can only live in a marine environment, but also those that lives revolve around the sea.

Marine organisms produce much of the oxygen we breathe and probably help regulate the earth's climate. Shorelines are in part shaped and protected by marine life, and some marine organisms even help create new land.

Marine biology covers a great deal, from the microscopic, including plankton and phytoplankton, which can be as small as 0.02 micrometers and are both hugely important as the primary producers of the sea, to the huge cetaceans (whales, dolphins and porpoises) which reach up to a reported 33 meters (109 feet) in length.

A large amount of all life on Earth exists in the oceans. Exactly how large the proportion is still unknown. While the oceans comprise about 71% of the Earth's surface, due to their depth they encompass about 300 times the habitable volume of the terrestrial habitats on Earth.

There is lot of Marine organisms in the sea can primarily classified as plants and algae, Marine invertebrates, Fish, Reptiles, Birds and marine mammals etc...Plant life is relatively rare undersea. Most of the niche occupied by plants on land is actually occupied by macroscopic algae in the ocean, such as Sargassum and kelp which are commonly known as seaweeds. These plants have adapted to the high salinity of the ocean environment. As on land, invertebrates make up a huge portion of all life in the sea. Fish have evolved very different biological functions from other large organisms (Wikipedia, 2007).

Comparatively Plants in the sea, especially seaweeds are much important than other organisms in the sea. They are harvested worldwide primarily for the extraction of chemicals that serve as gelling and thickening agents in foods, and for media used in medical and microbiological work, Industrial uses and directly as a vegetable (Bianchi et al., 1999).

2.2 Marine Algae (Seaweeds)

2.2.1 Introduction to marine algae

Most people have a general idea of what algae are. Algae (singular alga) are the aquatic photosynthetic organisms that are abundant in freshwater ponds, lakes and streams and in the ocean. Algae are called pond scums when they form floating mats or thin green films on the stems of aquatic plants. Some times algae become extremely abundant or "bloom" and discolor water bodies, i.e. red tides. Occasionally certain algae may be toxic to fish and invertebrates and become a nuisance during blooms. However, algae are often beautiful. Seaweeds, the algae growing in shallow marine areas and on exposed shorelines, are often delicately branched and strikingly colored in reds, greens and purples. Ingredients made from seaweeds are common in shampoos, toothpaste, ice cream, pastries, and beer. A wide variety of seaweeds are eaten directly as food, and some species of the red alga *Porphyra* are the delicious wrapping for sushi rolls.

The word "algae" derives from the Latin for "seaweed." The study of algae is called Phycology, based on the Greek word for seaweed, and scientist who study algae are called Phycologists. Algae are defined as photosynthetic organisms that lack flowers and the complicated structure of higher plants. These include a wide variety of organisms, from microscopic single cell to giant seaweeds more than 30 m long. Algae are mostly found in aquatic habitats, but some live on the surface of dry hot deserts or under Antarctic ice sheets. The color of algae range across the spectrum, from green to red, from yellow to black. These colors derive from small sac like structure in algal cells called chloroplasts, which contain the green pigments chlorophyll *a* and other accessory pigments whose yellow or red color may mask the green of the chlorophyll. These pigments all absorb light energy from the sun, which is by the plant to grow. The pigments also important in classifying algae in to Divisions, the major groups of algae that have different evolutionary histories (Readdie, M.D. et al., 2006).

Seaweeds are a fascinating and diverse group of organisms living in the earth's oceans. You can find them attached to rocks in the intertidal zone, washed up on the beach, in giant underwater forests, and floating on the ocean's surface. Although they have many plant-like features seaweeds are not true vascular plants; they are algae. Algae are part of the Kingdom Protista, which means that they are neither plants nor animals. Seaweeds are not grouped with the true plants because they lack a specialized vascular system (an internal conducting system for fluids and nutrients), roots, stems, leaves, and enclosed reproductive structures like flowers and cones. Because all the

parts of a seaweed are in contact with the water, they are able to take up fluids, nutrients, and gases directly from the water, and do not need an internal conducting system (Stewart, A., 1996).

2.2.2 Seaweeds around Sri Lanka

Sri Lanka has a rich marine algal flora along its coastal belt. The reported marine macro algal flora of Sri Lanka comprise of 396 species within 147 genera and 56 families. This number is based on only a handful of sporadic accounts and the marine algal diversity of Sri Lanka is still relatively poorly known (Lundsør, E., 2004).

In 1952 the Ceylon Fisheries Research station initiated the first systematic survey of the island's coastal waters for seaweeds with a view to utilizing those of economic importance. These investigations revealed that there were many species of algae on the west coast, but very few on the east coast. Extensive beds of seaweeds are found in Jaffna, Palk Strait, and Gulf of Mannar, Pearl bank off Silarathurai and along the southwest coast of Sri Lanka extending from Ambalangoda to Galle (Jayasuriya, P.M.A., 1990).

Along the Northern coast of Sri Lanka where the shores are shallow with sandy beaches, green algae are abundant in most places, and the area is also rich in brown algae. The North-East coast of Sri Lanka hosts few algal species, especially in Trincomalee even though the rocks appear to be good places for algal growth. However, along the Southern and Western coast of Sri Lanka well growing patches of diverse populations of algae (most of them red algae) can be observed at places where granite rocks and coral reefs are present. These reefs systems have evolved into extremely high levels of biological diversity including many uniquely specialized marine algae. The diversity and abundance of many algae show marked variations during the course of the year indicating the influence among others of the monsoonal changes of ocean characteristics on the reef flora. The monsoons play an important role in the algal vegetation of Sri Lanka. These reefs present a different picture during the two different monsoons. During the SW monsoon the sea is very rough and huge waves break on the edges of the reef. Due to violent waves many algae are uprooted or broken. Thus algal vegetation along these sites exhibit seasonal variations based on monsoonal effect (Lundsør, E., 2004).

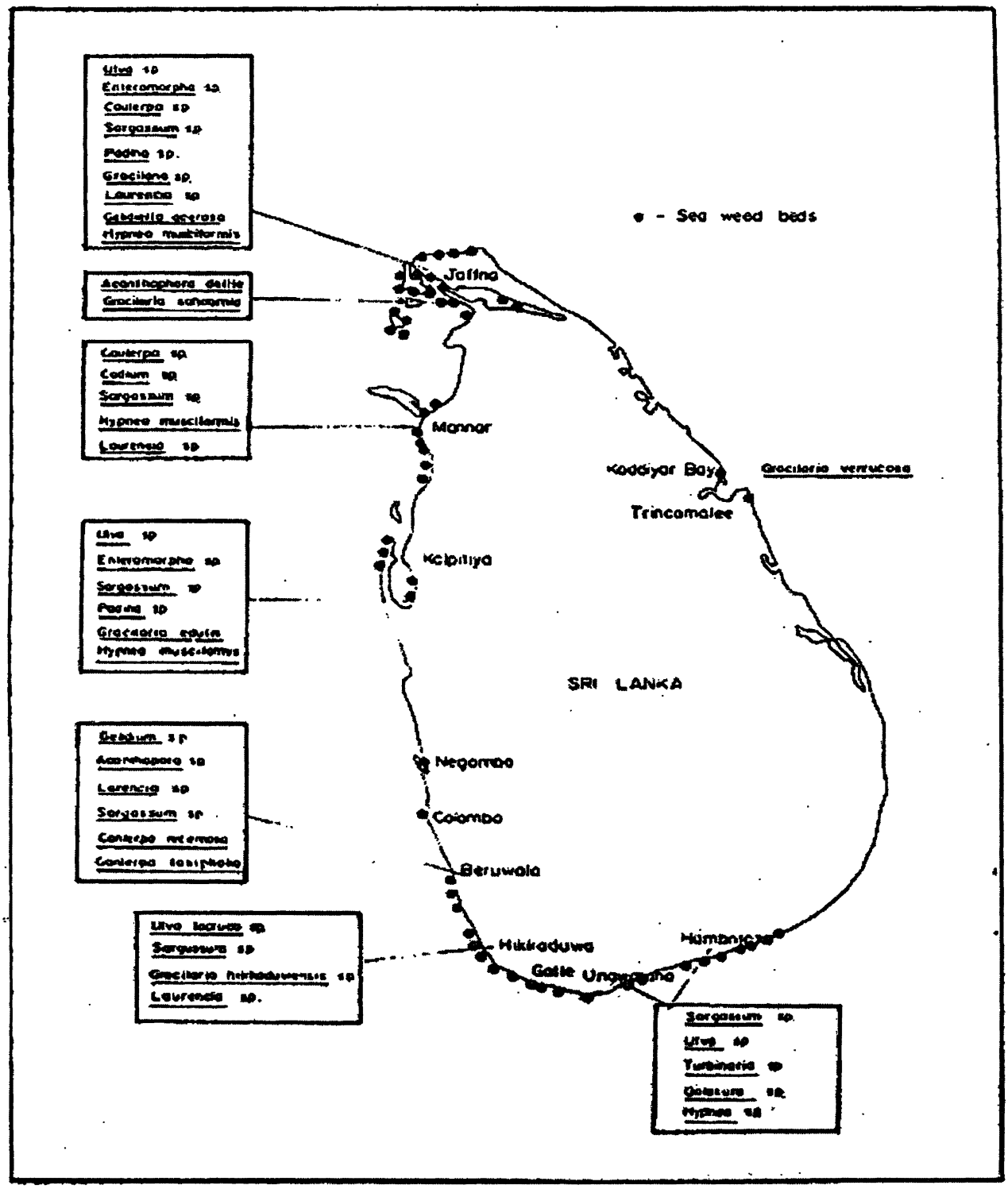

Fig. 2.1 The distribution of commercially important seaweed beds in the southern coast of Sri Lanka (Jayasuriya, P.M.A., 1990).

2.2.3 Habitats of seaweeds

Most marine algae require a stable surface on which to attach and grow, such as a rock or shell. Therefore, the best place to look for seaweeds is on a rocky shoreline. Many algae grow on solid rock, such a basalt or granite, while some send root like branches into softer limestone substrate called "coquina". Many types of seaweed that are torn loose from rocks can live for a while in the

flotsam on the ocean surface. Some grow as free-floating clusters of blades and air bladders, such as the mats of Sargassum that flourish in the Sargasso Sea in the Atlantic Ocean. Such "drift algae" are often tossed up on the beach, which makes the shore a good place to collect deep-water species without having to go scuba diving. Some seaweeds only grow on a particular surface, such as a species of red algae that grow only on the backs of sea turtles. Many types of algae grow on the surface of other algae or one of the rare flowering plants in the ocean and are called "epiphytes". A few red algae are parasitic, sending small branches into the tissue of the plant they grow upon and extracting nutrient from it.

Some algae are found only in mudflats or sandy bays near the mouth of rivers (even rivers that have dried up may still have large mudflats at their former mouth). Because these substrates are unstable, algae don't actually grow on the mud and sand itself. Instead, the algae grow on shells, isolated, rocks or portions of rocky reefs exposed above the surface of the mud or sand. These seaweeds must be resistant to scour or temporary burial during storms. In general, fever species of seaweeds grow in sandy or muddy habitats. If a river is actively draining into an estuary nearby, the salinity of the water may fluctuate, and the seaweeds living here must be able to tolerate less saline water. Similarly, algae growing in tide pools subject to evaporation must be able to tolerate somewhat higher salinities than those found in open water. Other factors that affecting seaweeds are light intensity, oxygen concentration in the water and wave action. The combination of these physical factors may favor the survival of some algae over others (Readdie, M.D. et al., 2006).

2.2.4 Algal form and structure

Some algae are unicellular; the whole plant is one cell, visible only with a microscope. The smallest macro algae are filaments, chains of cells that may be branched or unbranched. Slightly more complex are membranous algae, consisting of thin sheets one or two cells thick with flat or ruffled blades. Some algae grow as crusts, which may be soft and fleshy, leathery, or rock hard. Most complex are the erect branching forms with leaf-like or stem-like structure that give them the appearance of terrestrial mosses or flowering plants (Readdie, M.D. et al., 2006).

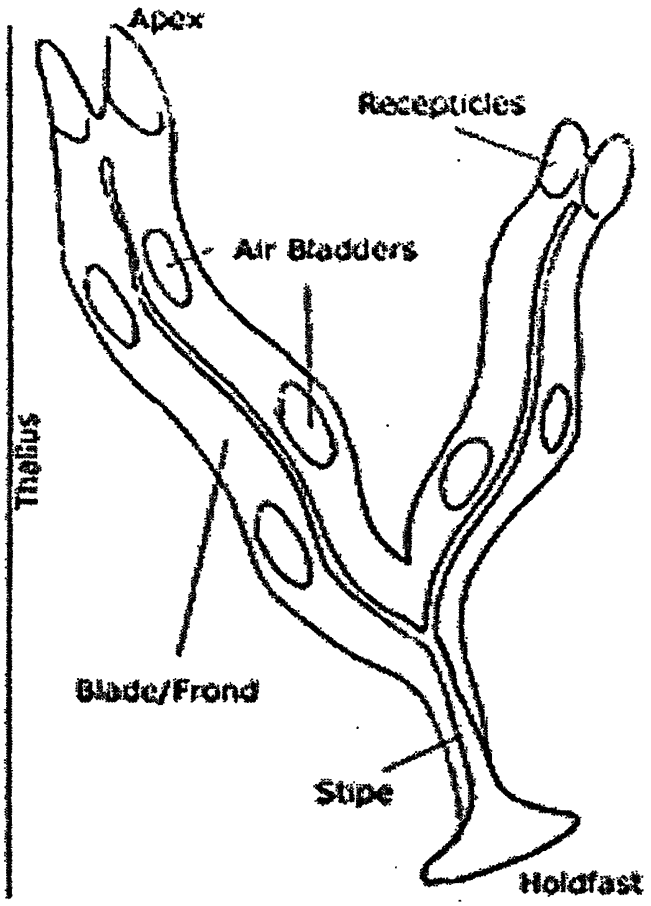

Fig. 2.2 Structure of Seaweed.

Instead of roots seaweeds have holdfasts, which attach them to the sea floor. A holdfast is not necessary for water and nutrient uptake, but is needed as an anchor. Holdfasts are made up of many fingerlike projections called haptera. There are 3 main types varying between species, the environment that they are found in and the size of the plant.

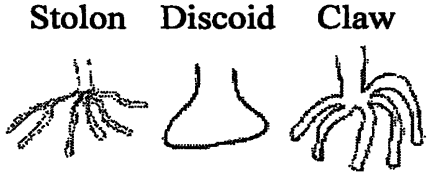

Fig. 2.3 Various types of Holdfasts (Davies, A., 2006)

The stalk or stem of seaweed is called a stipe. The function of the stipe is to support the rest of the plant. The structures of the stipe varies among the different types of seaweed; they can be flexible, stiff, solid, and gas-filled, very long (20 meters), short, or completely absent.

The leaves of seaweeds are called blades or fronds. The main function of the blades is to provide a large surface for the absorption of sunlight. In some species of seaweed, the blades also support

the reproductive structures of the seaweed. Some seaweeds have only one blade, which may be divided, while other species have numerous blades. The pattern of branching that the fronds exhibit can be grouped into four different types (summariesed below). Dichotomous branching is the most common, with species such as the fucoids have. Whorled generally occurs among the filamentous algae.

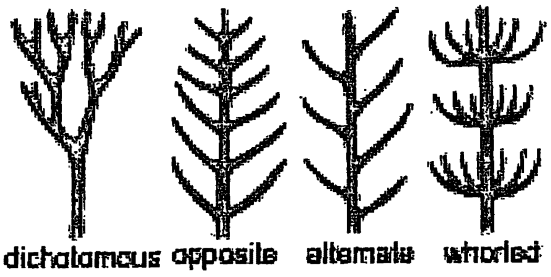

Fig. 2.4 Different types of Seaweeds (Davies, A., 2006)

Many types of seaweed have hollow, gas-filled structures called floats or pneumatocysts. These help to keep the photosynthetic structures of the seaweed buoyant so they are able to absorb energy from the sun. The proportion of gases in the pneumatocysts adjusts by equilibrating with the surrounding air or water. The pneumatocyst can hold O_2, CO_2 and CO.

Thallus is a way of saying the entire body of the plant, from top to bottom of the seaweed. The shape of the thallus allows us to simply classify the algae, for example the foliose algae are all sheet like, and very fragile. The filamentous algae are all thin lines of cells and the leathery macrophytes are large complex tough algae. So basically the thallus could be described as the unit that encompasses the entire plant, the reproductive organs, holdfast and all (Stewart, A., 1996 and Davies, A., 2006).

2.2.5 Ecology of seaweeds

Seaweeds play very important ecological roles in many marine communities. They are a food source for marine animals such as sea urchins and fishes, and are the nutritional base of some food webs. They also provide shelter and a home for numerous fishes, invertebrates, birds, and mammals.

Large seaweeds can form dense underwater forests, called kelp forests. These forests provide a physical structure that supports marine communities by providing animals with food and shelter. Kelp forests act as underwater nurseries for many marine animals, such as fish and snails. The lush blades form a dense forest canopy where invertebrates, fishes, birds, otters, and whales can find lots of tasty food and a good home. Beautiful sea slugs and kelp crabs can be seen on the blades and stipes of the seaweeds, while other small marine animals like worms find their homes

in the holdfasts. Kelp forests are a huge food source for sea urchins and other grazing invertebrates.

Seaweeds are affected by the physical characteristics of their environment. Because seaweeds absorb gases and nutrients from the surrounding water, they rely on the continual movement of water past them to avoid nutrient depletion. The constant motion of ocean water also subjects seaweeds to mechanical stress. Ocean waves and currents are sometimes strong enough to rip seaweeds right off the rocks! Seaweeds cope with mechanical stress by having a strong holdfast, a flexible stipe and blades, and bending towards the substrate as waves move over them.

Many types of seaweed live in rocky intertidal communities. Because they cannot get up and follow the water when the tide goes out, intertidal seaweeds are subjected to the stresses associated with exposure to air and weather conditions. To survive in the intertidal, seaweeds must be able to tolerate or minimize the effects of evaporative water loss and temperature and salinity changes. When exposed to air seaweeds lose water through evaporation. Some seaweeds can dry out almost completely when the tide is out, then take up water and fully recover when the tide brings water back to them. Seaweeds living in tide pools are exposed to changes in water temperature and salinity caused by weather conditions. On hot, sunny days the water in tide pools warms up and evaporates, which increases the salinity of the water. When it rains the opposite happens, the salinity of tide pool water decreases. On cold days, seaweeds can be damaged by freezing.

When the tide is out mobile intertidal animals must also try to minimize water loss. One way they do this is by seeking out a moist hiding place under some seaweed. As well as providing shelter for invertebrates, intertidal seaweeds are also a food source for grazing animals (Stewart, A., 1996).

The marine environment covers a wide thermal range (from the below freezing temperatures in Antarctic waters to about 350°C in deep hydrothermal vents), pressure range (1-1000 atm), nutrient range (oligotrophic to eutrophic) and it has extensive photic and non-photic zones (Jha and Zi-rong, 2004).

2.2.6 Algal Reproduction

Seaweeds reproduce by sexual reproduction and by vegetative growth. Instead of seeds, algae reproduce by variety of spores. Green and brown seaweeds produce microscopic single-celled spores capable of swimming by means of hair-like flagella attached to one end. Red algae also

produce single-celled spores, but these lack flagella and cannot swim. Spores are dispersed by ocean currents and turbulence until the spore attached to a suitable substrate and grows into the familiar larger form of the seaweed. Substrate where spores settle include exposed rocks, loose boulders, shells of living or dead mollusks, and frequently other algae.

Marine algae have life cycles unlike those of most land plants. Seaweeds sometimes have two different phases in the life cycle. For example, a small filamentous phase that grows in the algal turf may give rise to a larger, conspicuous vegetative phase (Readdie, M.D. et al., 2006).

2.2.7 General Characteristics

The best way for the beginners to learn about the diversity, Ecology, Morphology, and reproduction of marine plants is by observing them in the field. Marine plant can exhibit tremendous morphological variation among and within species; therefore it is important to pay close attention to differences on thallus appearance, blade shape, and branching pattern. In this way you can quickly become familiar with the amazing variety of forms that marine algae have to offer. The holdfast is often a diagnostic character for making a correct identification, particular with respect to marine algae.

The best way to preserve marine algae is by mounting them on herbarium paper. Specimens should be rinsed in seawater, shaken, and placed on a sheet of heavy rag paper. The paper should have a reference to your field notebook and a label with the species name, date, and location written in the bottom write hand corner. Use forceps or some other implement to spread the branches and blades to give the most representative and instructive presentation possible. The specimen may also be spread out onto the herbarium paper under a thin layer of water. This is especially useful for thin and delicate species. The paper is then slowly drawn out of the water, allowing the specimen to stick to the paper in the desired arrangement.

Scientists often call seaweeds as "benthic marine algae", which just means "attached algae that live in the sea" and there are about 10,500 species of seaweeds all over the world (Most of them are the green (1200 species), brown (2200 species) or red (6500 species)) (Guiry, M., 2007). From these species, approximately 6500 of species found in Indian region (900 species of green algae, 4000 species of red algae, and 1500 species of brown algae) (Khan, I.S., 2003).It is 60% from world distribution.

How ever, very few of the world's available seaweed species are used commercially. This may be because they cannot be harvested or cultivated on a commercially viable scale; or because their composition simply makes them unsuitable (Marsham et al., 2007). In 2003, It was estimated that approximately One millions tones of wet seaweed were harvested in 35 countries as a source of food; as a source of agar, alginates and carrageenan; as a fertilizer, as fuel and for use in cosmetics annually (McHugh, 2003).

The algae have chlorophyll and can manufacture their own food through the process of photosynthesis. Recently they are classified in the kingdom of protiste, which comprise a variety of unicellular and some simple multinuclear and multicellular eukaryotic organisms that have cells with a membrane-bound nucleus.
Almost all the algae are eukaryotes and conduct photosynthesis within membrane bound structure called chloroplasts, which contain DNA. The exact nature of the chloroplasts is different among the different lines of algae (Lenntech: algae description and types, 2006).

There are number of pigments found in seaweeds and they are varied widely among Green, Brown and Red algae. Among these pigments Chlorophyll-a, Chlorophyll-b, Chlorophyll-c, Xanthophylls such as Lutein, Violaxanthin, Fucoxanthin, β-carotene, Phycoerythrin and Phycocyanin etc... are important (Allen et al., 1962).

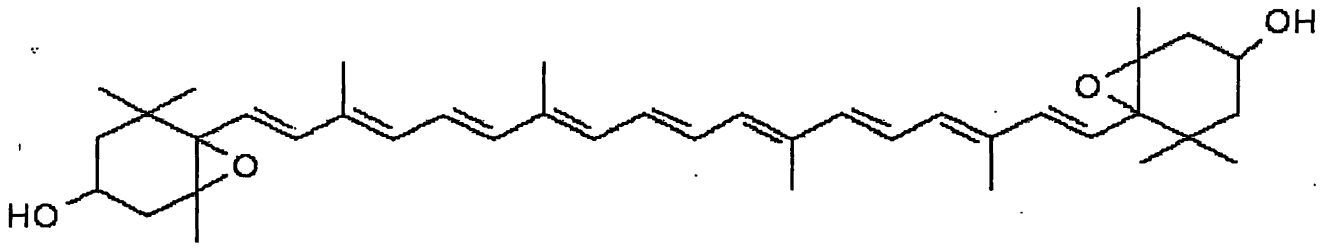

Fig. 2.5 Chemical Structure – Violaxanthin

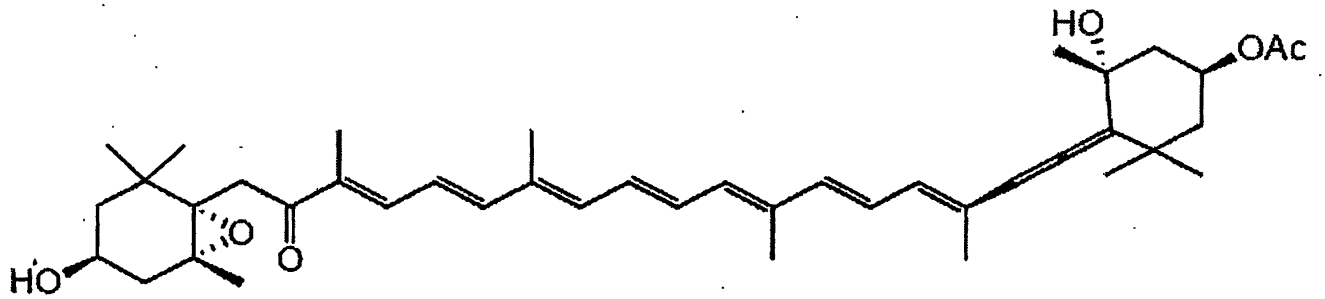

Fig 2.6 Chemical Structure – Fucoxanthin

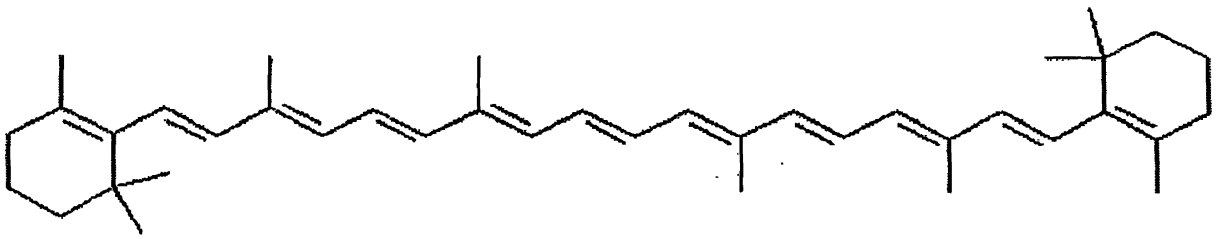

Fig 2.7 Chemical Structure – β-carotene

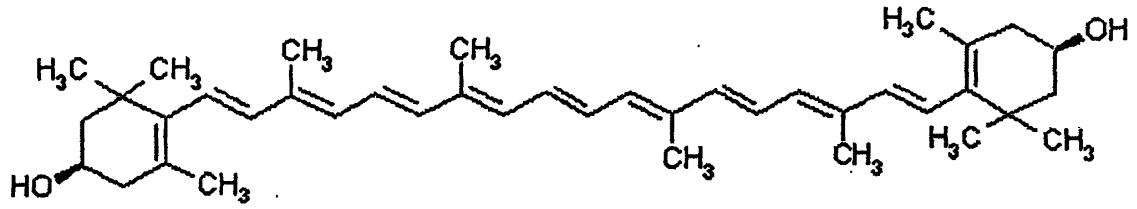

Fig 2.8 Chemical Structure – Lutein

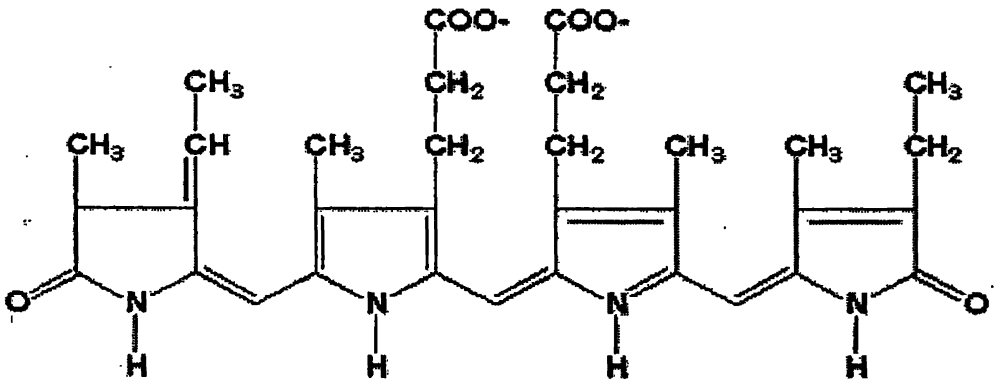

Fig 2.9 Chemical Structure – Phycocyanin

Sterols are a group of constituents of seaweeds with a possible biological activity and with a use for chemotaxonomy studies. There are typical studies for seasonal variations of sterol composition in algae have done by Kanias et al. Fucosterol and cholesterol characterizes the brown algae, cholesterol the red ones. Some other sterols have been isolated from the brown algae such as desmosterol, sargasterol, saringasterol etc...(Kanias et al., 1992).

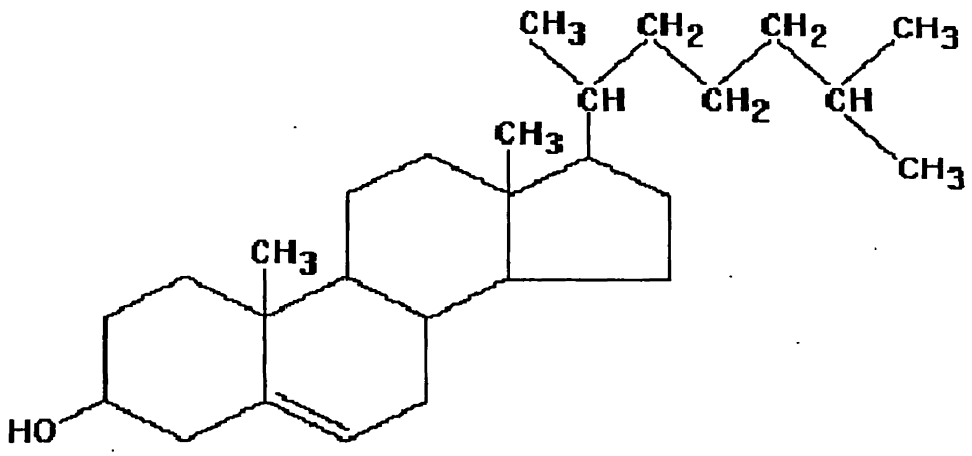

Fig. 2.10 Chemical Structure – Cholesterol

Moreover, it is well known that some trace elements play an important role for the growth and the maintenance of living organisms and a number of them have been recognized as essential for plants, animal and man. However, as far as there are no records for the essential concentration range of trace elements in lower plants and especially of sea plants. As trace elements can find Iron (Fe), strontium (Sr), Zinc (Zn), Cobalt (Co), Rubidium (Rb), Cesium (Cs), Scandium (Sc), antimony (Sb), europium (Eu), Chromium (Cr) and Thorium (Th) in most of the brown seaweeds (Kanias et al., 1992).

Seaweeds are a rich source of minerals, especially macro and micronutrients necessary for human nutrition; however, the nutritional properties of seaweeds are usually determined from their biochemical composition alone viz. proteins, carbohydrates, vitamins, amino acids etc... The mineral fraction of some seaweed even account for up to 40% of dry matter, however in some cases the mineral content of the seaweeds is recorded even higher than that of land plants and animal products. As minerals, can obtain Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu), Nickel (Ni) etc from seaweeds (Rao et al., 2007).
The protein content of seaweed varieties varies greatly and demonstrates a dependence on such factors as season and environmental growth conditions. The protein in algae contains all essential amino acids and all essential amino acids are available throughout the year although seasonal variations in their concentrations are known to occur (Dawczynski et al., 2007).

Carbohydrates which have been isolated from the brown seaweeds include low molecular carbohydrates such as mannitol, while laminaran, 'fucans' and alginates comprise a characteristic range of polysaccharides which have been found in all species of brown seaweeds investigated.

The sugar alcohol mannitol acts as a food reserve carbohydrate and also has a substrate for respiration. Laminaran is a β–D– (1→3) linked glucan found in all brown algae and occasionally in green algae. It is the food reserve material of the brown seaweeds which, unlike red and green algae, do not synthesize starch like polysaccharides. 'Fucans' are water soluble and present in the intercellular tissue of brown seaweeds. It is also found in the mucilage which exudes from the surface of fronds. Alginic acid is a mucilaginous polyurinide which is an important cell wall constituent of the brown seaweeds, where it occurs as a mixed salt of sodium, calcium and magnesium (De Silva and Savitri Kumar, 1988).

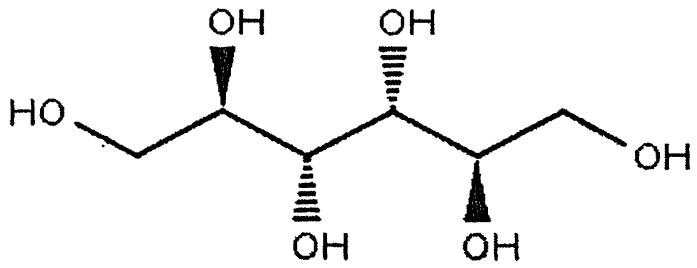

Fig. 2.11 Chemical structure – Mannitol

2.2.8 Metabolites from Seaweeds

All organisms needs to transform and inter convert a vast numbers of Organic compounds to enable them to live, grow and reproduce. They need to provide themselves with energy in the form of Adenosine triphosphate, and a supply of building blocks to construct their own tissues.

The many chemical reactions involved in these activities are summarized as metabolism and the participating compounds are metabolites. The smallest metabolite is the proton, but the term excludes the very large macromolecules and refers to compounds having molecular masses with an upper limit of about 1500 Daltons.

Metabolites are classified into two broad types, primary and secondary. Primary metabolites are essential to growth and life in all living systems, and are formed by a limited number of metabolic reactions. Primary metabolites serve as building blocks for synthesis of macromolecules, proteins, nucleic acids, carbohydrates and lipids. Secondary metabolites are not essential to the life of the producing organism and are formed from primary metabolites. Many of the secondary metabolites enhance the survival fitness of the organism and may serve for example as chemical weapons used against bacteria, fungi, insects and large animals. Most of the natural products of interest to the pharmaceutical industry are secondary metabolites, but there is also growing interest in

products of primary metabolites such as various marine lipids, enzymes and complex heteropolysaccharides (Gudbjarnason, S., 1999).

Marine algae are one of the largest producers of biomass in the marine environment. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against other settling organisms. These active metabolites, also known as biogenic compounds, produced by several species of marine macro- and micro-algae, have antibacterial, antialgal, antimacrofouling and antifungal properties, which are effective in the prevention of biofouling, and have other likely uses, e.g. in therapeutics. The isolated substances with potent antifouling activity belong to groups of fatty acids, lipopeptides, amides, alkaloids, terpenoids, lactones, pyrroles and steroids. These biogenic compounds have the potential to be produced commercially using metabolic engineering techniques. Therefore, isolation of biogenic compounds and determination of their structure could provide leads for future development of, for example, environmentally friendly antifouling paints (Punyasloke and Phillip, 2004).

The mechanism by which marine algae show resistance to pathogenic microorganisms remains poorly understood. From the organic extract of the brown alga *Taonia atomaria*, two novel cyclized meroditerpenoids atomarianones were isolated and both metabolites were found to exhibit significant cytotoxic activity against two lung cancer cell lines (Abatis et al., 2005). Methyl 2-[propanamide-2'-methoxycarbonyl] benzoate, fucosterol, trans-phytol and p-formylphenol were isolated from a methanolic extract of *Jolyna laminarioides*. Methyl 2-[propanamide-2'-methoxycarbonyl]-benzoate exhibited chymotrypsin inhibitory activity and also found to be active against *Escherichia coli* and *Shigella boydii*. Fucosterol exhibited antifungal activity against *Curvularia lunata*, *Stachybotrys atra* and *Microsporum canis* (Atta-Ur-Rahaman et al., 1997). Sterols are another important metabolite found in Marine algae, especially in Brown algae and among these sterols, fucosterol, saringosterol and 24-ketocholesterol are frequently found (Kurata et al., 1990).

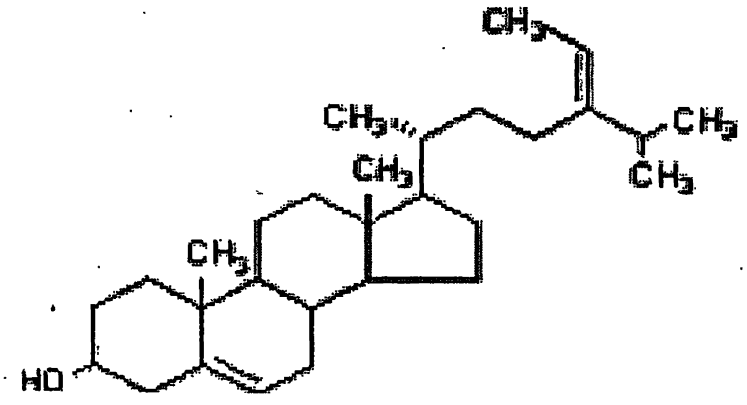

Fig. 2.12 Chemical Structure – Fucosterol

They contain all major and minor plant nutrients as well as biocontrol properties and contain many organic compounds such as auxins, gibberellins and precursor of ethylene and betaine which affect plant growth. Liquid concentration of brown algae *Ecklonia maxima* significantly reduced the root knot infestation and increased growth of tomato plant. Antimicrobial activity of Canary species of Phaeophyta and Chlorophyta has been reported. Seaweeds occurring at Karachi, Pakistan coast have also shown cytotoxic, nematicidal and fungicidal hypoglyceamic and antibacterial activities. Soil amendment with brown seaweeds *Stoechospermum marginatum* and *Sargassum tenerrimum* with or without rhizobia significantly reduced root knot nematode (*Meloidogyne javanica*) and root infecting fungi infections (Sultana et al., 2005)

Presence of anticoagulant activity in brown algae was first reported in 1941, where *Laminaria* showed anticoagulant effect, its active compound being located in hold fasts. Around 60 brown algal species are identified to have blood anticoagulant properties. The anticoagulant components of brown marine algal extracts are found in a group of polysaccharides, more commonly referred to as 'fucans'. In brown algae, fucoidan has been the most extensively-studied fucan. The initial studies described significant *in vitro* and *in vivo* activity of fucoidan from *Fucus vesiculosus*. Furthermore, it was found that no toxic symptoms were displayed. The most active fucoidan fractions predominantly consisted of sulphated fucose residues (Shanmugam and Mody, 2000).

The cell walls of seaweeds contain abundant matrix polysaccharides formed by neutral and acid sugars that are also found in terrestrial plants. In addition, most seaweed also contains sulphated polysaccharide; where as most terrestrial plants do not. The way the sugars are linked and the presence of sulphated groups allow the formation of a vast number of molecules with different shapes and biological properties including antiviral, anticoagulant, antitumour, and immunomodulatory activities in mammals.

2.2.8.1 Agar

Agar is usually obtained from some Red algae and the agar producing algae are generally called as agarophytes. Agar is extensively used in Sri Lanka and annually large quantities of these products are imported. Agar is mainly used in confectionaries as a substitute for gelatine. It is also used in culture medium for the growth of microorganisms like bacteria and fungi. Most of the agarophytes found in Jaffna peninsula normally all over the west coast of Sri Lanka (Arumugam et al., 1981). More than 50 species of red algae have been use as raw material in the manufacture of agar. Most agars are extracted from species of Gelidium and Gracilaria. Gelidium species are small, slow growing plants, and while cultivation in ponds and tanks is possible, to date it has not been economically viable. Gracilaria cultivation is widespread, and several methods are used (McHugh, 2003).

The original structure of agar was believed to be a simple sulphated poly glactose. How ever it was found that agar is consisted of at least two separate polymers that could be fractionated. One was called agarose and the other agaropectin. Those are polysaccharides. Agarose is a linear chain of alternating neutral sugar residues- mainly 1, 3 linked D- galactose and 1, 4 linked 3,6-anhydrous L-glactose residues. Agaropectin consists mainly of D-galactose, 3, 6-anhydro-L-galactose, some ester sulphate and D-glucuronic acid. It has many of the structural features of agarose.

Agar is insoluble in cold water but readily dissolve in boiling water, and set to a firm gel at concentration as low as 0.5%. It is a valuable colloidal substance because of its hydrophilic nature and its high gel strength. These two important properties are responsible for its wide use in the food, pharmaceutical and textile industries as a thickening, emulsifying, stabilizing and gelling agent. It is also used in medical and bacteriological laboratories as a culture medium for microorganisms. In Japan, the largest amount of agar is extracted mainly from plants of the genus *Gelidium* (Dantanarayana et al., 1981).

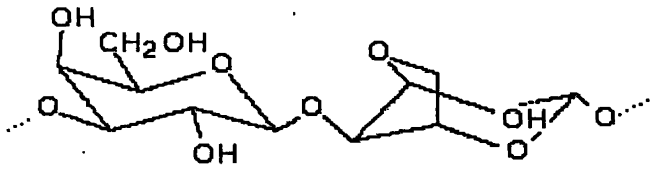

Fig. 2.13 Chemical Structure – Agar repeat unit

2.2.8.2 Carrageenin

Carrageenans or carrageenins are a family of linear sulphated polysaccharides extracted from the cell walls of marine red algae. There are several carrageenans, differing in their chemical structure and properties, and therefore in their uses. The carrageenans of commercial interest are called iota, kappa and lambda. The largest producer is the Philippines, where cultivated seaweed produces about 80% of the world supply. Carrageenin is use to produce many products such as Desserts, ice cream, milk shakes, sauces - gel to increase viscosity, processed meat - Substitute fat to increase water retention and increase volume, Shampoo and cosmetic creams – thickener, Pharmaceuticals - used as an inactive excipient in pills/tablets etc...(Parekh et al., 1988)

Different seaweeds produce different carrageenans. Carrageenans are linear polymers of about 25,000 galactose derivatives with regular but imprecise structures, dependent on the source and extraction conditions. κ-carrageenan (kappa-carrageenan) is extracted from *Kappaphycus alvarezii* and having structure of -(13)-β-D-galactopyranose-4-sulfate-(14)-3,6-anhydro-α-D-galactopyranose-(13). ι-carrageenan (iota-carrageenan) is extracted from *Eucheuma denticulatum* and λ-carrageenan (lambda-carrageenan) is extracted from *Gigartina pistillata* (Michel et al., 2001).

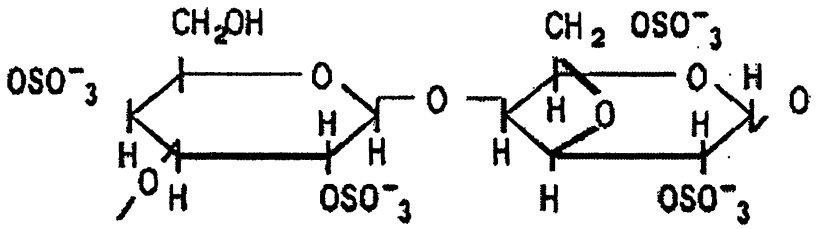

Fig. 2.14 Chemical Structure – Carrageenin

2.2.8.3 Alginates

Alginate, sometimes shortened to "algin", is present in the cell walls of brown seaweeds as the calcium, magnesium and sodium salts of alginic acid, and it is partly responsible for the flexibility of the seaweed. A high quality alginate forms strong gels and gives thick aqueous solutions. "Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself (McHugh, 2003).

Alginic acid is important commercial polysaccharides that could be obtained from marine algae. Algin is usually obtained from some brown algae and the alginic acid producing algae are generally called alginiphytes. No systematic studies have been made on the content of the alginic acid and agar present in the marine algae found in Sri Lanka (Arumugam et al., 1981).

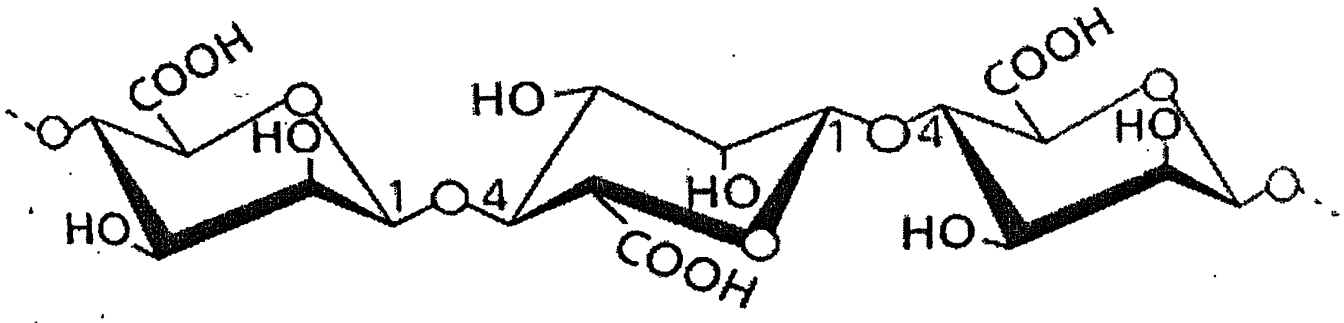

Fig. 2.15 Chemical Structure – Alginic Acid

There are two different ways of recovering the alginate. The first is to add acid, which causes alginic acid to form; this does not dissolve in water and the solid alginic acid is separated from the water. The second way of recovering the sodium alginate from the initial extraction solution is to add a calcium salt (McHugh, 2003).

2.2.9. Uses of Seaweeds

Seaweeds have been used in the world for over 1000 years as food, animal fodder, fuel, manure and for dyeing, medicinal purposes, the making of kelp and for various minor uses and numerous references on this can be found in the extensive literature written during the past eight centuries.

2.2.9.1. Seaweeds for human consumption

For several centuries there has been a traditional use of seaweeds as food in China, Japan and the Republic of Korea. According to the literature, *Rhodymenia palmata* called "sol" has been known to be edible since at least the year 961. The most important use of algae is that they are the "Primary producers of organic matter in aquatic environment because of their photosynthetic activity". Animal life in aquatic environment mainly depends on algae because they form the primary source of energy and food for them. In aquatic ecosystem the algae constitute the main part of the food chain. Because of their photosynthetic activity they continuously oxygenate their surrounding aquatic environment, which is beneficial directly to the other aquatic organisms.

In the world, peoples are using 1,000 species of seaweeds, mostly Phaeophyceae and Rhodophyceae, as food. These weeds are more important as a food due to presence of minerals, vitamins, carbohydrates and proteins, either in their cell wall or in their cytoplasm. Seaweeds have an excellent nutritional content, mainly of protein, carbohydrate, vitamin B, B2, A and C and lots of trace elements and minerals, most prominent of these is iodine. Another plus is that they are low in calories and re suitable for the ever-increasing vegetarian market. Most popular algae that use as a food are Nori (*Porphyra* species), Kombu (*Laminaria* species), and Wakame (*Undaria pinnatifida*) (Hallsson, 1961) Most recently seaweeds have been utilized in Japan as raw materials in the manufacture of many seaweeds food products such as jam, cheese, wine, tea, soup and noodles (Rao et al., 2007)

2.2.9.2. Feeding of Seaweeds to domestic animals

In Iceland Marine algae such as brown algae and red algae are commonly use as a animal food for cattle, sheep and horses. Seaweed-meals also increase the butterfat content of the milk in feeding cattle's. For industrial preparation of animal food use *Ascophyllum nodosum*.

2.2.9.3. Seaweed industrial gums

The term "industrial gums" is a generic name for a range of products that are either manufactured artificially or extracted from animals or plants and used to achieve various levels of viscosity. These all Gums fall into three categories: alginates (derivatives of alginic acid), agars and carrageenans.

Tab. 2.1 Production & value of international seaweed gums market, 1995.

Seaweed gum	Total (t)	Price ($ per kg)	Total value ($ million)
Agars	10,161	20	203
Carrageenans	25,403	8	203
Alginates	>25,000	6	150
Total	>61,000	-	560

- **Alginates**

  Alginates are cell-wall constituents of brown algae (Phaeophycota). Alginates, especially sodium alginate, are widely used in the textile industry and calcium alginates in Pharmaceutical industry. Other uses include glazing and sizing paper, special printers' inks, paints, cosmetics, insecticides, and pharmaceutical preparations (Guiry, 2007).

- **Agar**

  Agar, a general name for polysaccharides extracted from certain kinds of red algae, is built up of alternating D- and L- galactopyranose units. The best quality agar is extracted from species of the red algal genera *Pterocladia*, *Pterocladiella* and *Gelidium* and agar quality is seasonal in *Pterocladiella* species, being low in the colder months and high in the warmer. Major agar producers are Argentina, Canada, Chile, China, France, India, Indonesia, and Japan (Guiry, 2007).

- **Carrageenin**

  Carrageenin is a general name for polysaccharides extracted from certain kinds of algae which are built up, in contrast to agar, from D-galactopyranose units only. Major carrageenin producers are Philippines, Indonesia, Malaysia, and Tanzania (Guiry, 2007).

2.2.9.4. Medicinal uses

In Europe and North America, many claims have been made for the effectiveness of seaweeds on human health. It has been suggested, amongst other things, that seaweeds have curative powers for tuberculosis, arthritis, colds and influenza, worm infestations, and may even improve one's attractiveness to the opposite sex. Some kelp may have polysaccharides that apparently reduce the incidence of breast cancer. *Saccharina* and *Sargassum* have been used in China for the treatment of cancer (Guiry, 2007).

- **Kunbu** (*Saccharina* and *Ecklonia*) (*Kombu* in Japan)
    - Essence and Flavor: Salty, Cold
    - Channel Entered: Liver, Stomach, Kidney
    - Actions: Softens hardness, disperses accumulation, resolves phlegm
    - Applications: Scrofula, goiter, tumor, edema, accumulation and testicular pain

- **Haizao** (*Sargassum*) (*Hiziki* in Japan)
    - Essence and Flavor: Bitter, Salty, Cold
    - Channel Entered: Liver, Stomach, Kidney
    - Actions: Disperses accumulated phlegm, disperses goiter and tumor
    - Applications: Scrofula, goitre, tumor, edema, testicular pain and swelling

- **Zicai** (*Porphyra*) (*Nori* in Japan)
    - Essence and Flavor: Sweet, Salty, Cold
    - Channel Entered: Lung
    - Actions: Resolves phlegm, softens hardness, dispels heat, promotes diuresis
    - Applications: Goiter, beriberi (leg swelling), edema, urinary infection, sore throat

2.2.9.5. As a source of minerals

Seaweeds are rich source of minerals, especially macro and micronutrients necessary for human nutrition. The genus *Porphyra*, traditionally known as *nori*, in Japan *kim* and in Korea *zicai* in China, is a popular delicacy, due its rich content of protein, Vitamins, minerals and dietary fibres. This algae is also reported to contain iodine, bioactive substances and anti fungal compounds of therapeutic value.

Seaweeds are rich source of Sodium (Na), Iron (Fe), Manganese (Mn), Potassium (K), Calcium (Ca) and Magnesium (Mg). Lead, Chromium, Cobalt, Zink, Mercury, Copper, Arsenic, Nickel and Cadmium are found in trace amounts (Darcy-Vrillon, 1993).

2.2.9.6. Fertilizers and soil conditioners

According to research, the colloidal seaweed extracts have been reported to contain several substances with properties helpful for plant growth and yield. Trace elements inherent in seaweed extracts are especially effective in foliar feeding to all types of plants. It increases in weight or yield of plant and plant parts. It is now regarded as a valuable supplement to soil feeding in many circumstances, such as correcting iron deficiencies and compensating for lower nutrient uptake in very wet or dry summer spells (Sivasankaria et al., 2006).

2.2.9.7. Uses of seaweeds as a fuel

Where peat was scarce dried seaweeds have been used as fuel up to the beginning of this century. The main species used was *A. nodosum* cast or cut. Seaweeds can use as renewable source of methane (natural gas) and there are many studies to seaweed convert to methane by a process of anaerobic fermentation. Methane from marine biomass is a long-term project and research and development have been scaled down, probably to be revived when a crisis threatens in natural gas supplies (Hallsson, 1961).

2.2.9.8. Miscellaneous Uses

Seaweeds have number of minor beneficial aspects in all over the world. They are varying with verities, composition, needs of users and culture of the users etc... There are many records for the use of seaweeds in cosmetics industry and waste water treatment. There are two main areas where seaweeds have the potential for use in wastewater treatment. The first is the treatment of sewage and some agricultural wastes to reduce the total nitrogen- and phosphorus-containing compounds before release of these treated waters into rivers or oceans. The second is for the removal of toxic metals from industrial wastewater (McHugh, 2003).

2.2.10 Negative aspects of Seaweeds

If somebody eat seaweeds in high amount daily for a long time, he get excess of minerals and other nutrients to the body. When having excess of some metals and mineral, like Arsenic, mercury, etc..., it cause to inherent diseases. There are some small marine algae having poison matters, when these plants eat by fishes, these things are accumulate in their bodies. If somebody eats fish like these, it may cause to sudden death.

Some marine algae cause to skin irritation, when they are touch with skin. Stinging seaweed disease is a skin irritation caused by direct exposure to a poisonous type of algae named *Lyngbya majuscula.* When large amount of algae are decaying, some toxins can be liberate. It may be reason for death of some fish, having their habitat with algae (Wheeler et al., 1979).

2.2.11 Seaweed Classification

Seaweeds are classified into three major groups; the green algae, the brown algae, and the red algae. Placement of seaweed into one of these groups is based on the pigments and coloration existing in the plant. Other seaweed features that are used to classify algae include: cell wall composition, reproductive characteristics, and the chemical nature of the photosynthetic products. Plant structure, form and shape are additional characteristics used to classify seaweed. All of these are put in to the group protista. Another group, the blue-green algae, is the cyanobacteria. Some authorities do not consider the blue-green algae to be true algae because they are prokaryotes, not eukaryotes (Wellsa et al., 2007).

Green Algae: - Green algae are the algae most closely related to plants. They have the same pigments (chlorophyll a and b and carotenoids), the same chemicals in their cell walls (cellulose), and the same storage product (starch) as plants. Green algae may be unicellular or form filaments, nets, sheets, spheres, or complex mosslike structures. There are both freshwater and marine species. Some species of green algae live on snow, or in symbiotic associations as lichens, or with sponges or other aquatic animals. Edible green algae include Chlorella and sea lettuce. There are at least seventeen thousand species of green algae (Wellsa et al., 2007).

Red Algae: - Red algae are almost exclusively marine and include many edible and economically important species, including nori and laver. Red algae are also the source of carageenan and agar, which are used as food thickeners and stabilizers. Red algae are mostly large, complex seaweeds. There are four thousand to six thousand species of red algae around the world (Wellsa et al., 2007).

Brown Algae: - Brown algae are almost exclusively marine and include the largest and most complex seaweeds. Kelp, for example, may be more than 60 meters (200 feet) tall, and forms dense underwater forests. Other important brown algae include the rockweeds and Sargassum. There are about fifteen thousand species of brown algae (Wellsa et al., 2007).

2.3 Brown Algae

The brown algae include some of the largest and most complex seaweeds: the kelps, wracks and sargassums. Brown algae belong to the Phylum Phaeophyta and are particularly common in the temperate zones of the world, although many species of sargassum grow in warmer waters (Vithanage et al., 1983).

Brown algae are all multi-cellular, and are found in a variety of different physical forms including crusts, filaments, and large elaborate kelps. Like all photosynthetic organisms, brown algae contain the green pigment chlorophyll. They also contain other gold and brown pigments, which mask the green color of chlorophyll. The dominant pigment found in brown algae is called fucoxanthin, and it reflects yellow light. Because of their combination of pigments, the coloration of brown algae ranges from light olive green or golden, to very dark brown. Most brown algae live in the intertidal or shallow subtidal zone, and they are most abundant in the colder oceanic waters of the Northern Hemisphere (Stewart, A., 1996).

2.3.1 Importance of Brown Algae

Humans use brown seaweeds in numerous ways, and algae are becoming very important commercially for food, cosmetics, and pharmaceuticals and in the sciences. Many tasty types of kelp are harvested from wild populations and also grown in commercial kelp farms. Two unique types of compounds are found in brown algae, algin and fucans, which are used in the manufacturing of consumer goods. Algin is found within the cell walls of brown algae, and it is an emulsifier used in food products. Fucans are the slimy stuff found on kelps, and have potential medicinal uses. Brown algae are also collected, treated, and sold as a fertilizer for terrestrial agriculture (Stewart, A., 1996).

2.3.2 Phaeophyta class

Brown algae is classifies as Phaeophyta commonly in to a one class, having 14 orders. These things can scientifically classify as follows. Domain: Eukaryota, Kingdom: Chromalveolata, Phylum: Heterokontophyta and Class: Phaeophyceae.

2.3.4 *Padina pavonica*

Padina pavonica is a common brown alga found in all over the world. It is also known as peacock tail. The frond is thin and leafy, flattish and entire when young, but often concaves, or almost funnel shaped in mature specimens, with a lactiniate or irregularly lobed margin. The inner (or upper) surface is covered in a thin coating of slime, and the outer (or lower) surface is banded with zones of light brown, dark brown and olive green. Small, fine hairs form concentric lines, 3-5 mm apart, from the outer margin continuing down the outer (coloured) surface of the fronds.

- **Scientific classification**

Kingdom	:	Protista
Division	:	Heterokontophyta
Class	:	Phaeophyceae
Order	:	Dictyotales
Family	:	Dictyoptaceae
Genus	:	*Padina*
Species	:	***P. pavonica***

- **Key identification features**

  - Concave or almost funnel-shaped frond.
  - Frond thin with irregular lobed margin.
  - Upper surface has thin layer of slime.
  - Lower surface banded with zones of brown and green.
  - Concentric lines of small fine hairs on lower surface.

There are limited data on the chemical composition of the algae from the Genus *Padina*. Fatty acids, containing 14-22 carbon atoms have been identified in different *Padina* species. Significant differences in sterol composition were found within the different *Padina* species. Fucosterol predominated in *Padina gymnospora* and no cholesterol found. Fucosterol is also the main sterol in *Padina crassa*. In *Padina vickersiae* and *Padina pavonica* (34%) the main sterol is cholesterol. But according to the region found algae species this sterol composition is greatly varying (Kamenarska, et al., 2002).

Few terpinoids have been found in Padina species. Halogenated terpinoids were identified in *Padina tetrastromatica*. Chemical examination of Petroleum ether fraction of *Padina tetrastromatica* yielded several fatty acids, sterols and halogenated terpinoids (Parameswaran, et al., 1996)

There are lots of photosynthetic pigments in *Padina pavonica* and 14 of them were identified by Hegazi et al. Chlorophyll c_1, c_2, Fucoxanthin, Fucoxanthol, Flavoxanthin, Diatoxanthin were the most typical pigments in *Padina pavonica* (Hegazi, et al., 1998).Sterols and Lipids are important constituents of the cell membranes and are responsible for many of

the cell functions. Volatile compounds often contain biologically active compounds (Acids- 9.98%, esters- 35.57%, Phenols- 3.14%, Alcohols-18.22%, Aldehyde-4.21%), some of them with allopathic activity. Polar fractions, extraction with n-butanol, are very complex and there are limited data on their composition. *Padina pavonica* show antibacterial and antifungal activity against *Staphylococcus aureus* and *Candida albicans* (Kamenarska, et al., 2002).

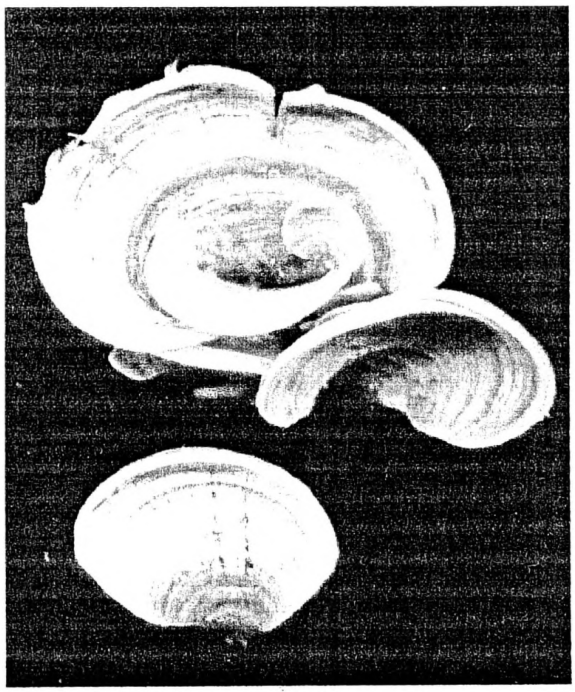

Fig. 2.16 *Padina pavonica*

2.3.5 *Ulva lactuca*

- **Scientific classification**

Domain	:	Eukaryota
Kingdom	:	Protista
Phylum	:	Chlorophyta
Class	:	Ulvophyceae
Order	:	Ulvales
Family	:	Ulvaceae
Genus	:	*Ulva*
Species	:	***U. lactuca***

Ulva lactuca, also known as Sea Lattuce is a thin flat green alga growing from a discoid holdfast. The margin is somewhat ruffled and often torn. It may reach 18 cm or more long though generally much less and up to 30 cm across. The membrane is two cells thick, soft and translucent, and grows attached, without a stipe, to rock via a small disc-shaped holdfast. Green to dark green in color this species in the Chlorophyta is formed of two layers of cells irregularly arranged, as seen in cross section. This Chlorophyte is a sheet forming alga composed of two layers or cells, as seen in cross section here. Ulva, among other green algae, is very prolific in areas were there are lots of nutrients available (Wikipedia, 2007).

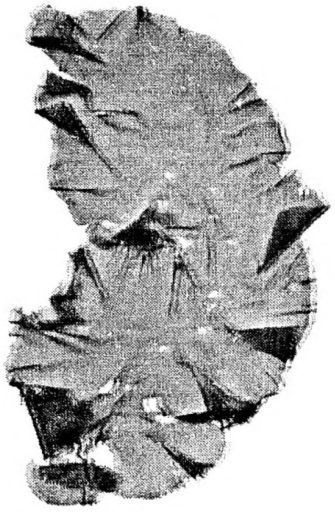

Fig. 2.17 *Ulva lactuca*

2.4 Chromatographic Techniques

Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.

Chromatography may be preparative or analytical. Preparative chromatography seeks to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography normally operates with smaller amounts of material and seeks to measure the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Definition: chromatography is a physical method of separation, in which the components to be separated are distributed between two phases, one of these phases constituting a stationary bed of large surface area, the other being a fluid that percolates through or along the stationary bed (Day and Underwood, 1998).

2.4.1 Thin Layer Chromatography

TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate).

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action (Day and Underwood, 1998).

2.4.2 Column Chromatography

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture.

The mixture to be analyzed by column chromatography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column.

Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called flash chromatography (Day and Underwood, 1998).

2.5.3 HMQC Spectroscopy and HMBC Spectroscopy

Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) are 2-dimensional inverse H, C correlation techniques that allow for the determination of carbon (or other heteroatom) to hydrogen connectivity. HMQC is selective for direct C-H coupling and HMBC will give longer range couplings (2-4 bond coupling). Heteronuclear Multiple Quantum Correlation is an experiment that identifies protons with their directly bound carbons. Heteronuclear Multiple Bond Correlation is an experiment that identifies proton nuclei with carbon nuclei that are separated by more than one bond (Akitt and Mann, 2004).

CHAPTER 3
MATERIAL & METHODOLOGY

3.1 Materials

3.1.1 Apparatus

Plant Materials of *Padina pavonica*

Oven drier

Model 4MXGR Mechanical grinder

USC 1700D Ultra Sonicator

Heidolph Laborota 4000 Rotary evaporator

Merck Art 60765 Silica gel Pre coated Aluminium sheets

Merck Art 77340 Silica gel

Fluka 60741 Column

Precisa 180A Electrical balance

Spectroline Model CM-10 Fluorescence analysis cabin

Varian 300NMR Spectrophotometer

3.1.2 Solvents

Hexane

Dichloromethane

Methanol

Ethyl Acetate

Distilled water

3.2 Methodology

3.2.1 Sample collection and preparation of Marine algae

The brown marine algae *Padina pavonica* were collected in mostly young stage freshly, from Arugam Bay shores (6°50'45"N 81°50'3"E), near Potuvil in Ampara District, Eastern province of Sri Lanka. They were identified by Ms. Rasika Premaratne, Institute of Fundamental Studies by comparison with specimens at the National Herbarium Peradeniya botanical garden. Then the plant materials were washed with fresh water for several times. After draining water from plants they were kept in a shade for several days for air dried. A voucher Specimen was deposited by fixing it in 3-5% buffered Formalin

at Natural Product Laboratory of Institute of Fundamental Studies, Kandy, Sri Lanka. Then dried *Padina pavonica* sample (450g) was powdered using a mechanical.

3.2.2 Methanolic extract of *Padina pavonica*

Powdered *Padina pavonica* were placed to a large flask and sonicated using an ultrasonicator to extract plant material to MeOH. Extractions were done for three times (30min. x 3) and filtered through cotton wools to get the extract. Methanol extract was evaporated using a rotary evaporator with vacuum to dryness at 45 ^{0}C. Finally obtained 3.0g of the extract. TLC was carried out for crude extract for check the distribution of the content of the extract.

3.2.3 Slurry preparation

The slurry was prepared using 3.0g of crude plant extract and three times of silica gel (0.063-0.200mm) by dissolve in Hexane and evaporating using a rotary evaporator with vacuum at 45 ^{0}C.

3.2.4 Column running and Compounds Isolation

The prepared slurry of *Padina pavonica* was chromatographed using silica gel (0.063-0.200mm) column chromatography. The column was packed with 100% hexane, which has least polarity, 25g of silica gel. After packing the column the height of the column was 25cm with 2cm diameter. Then the slurry was placed to top of the column and the column was run using following solvents by increasing the polarities.

- Hexane
- Dichloromethane
- Methanol
- Distilled Water

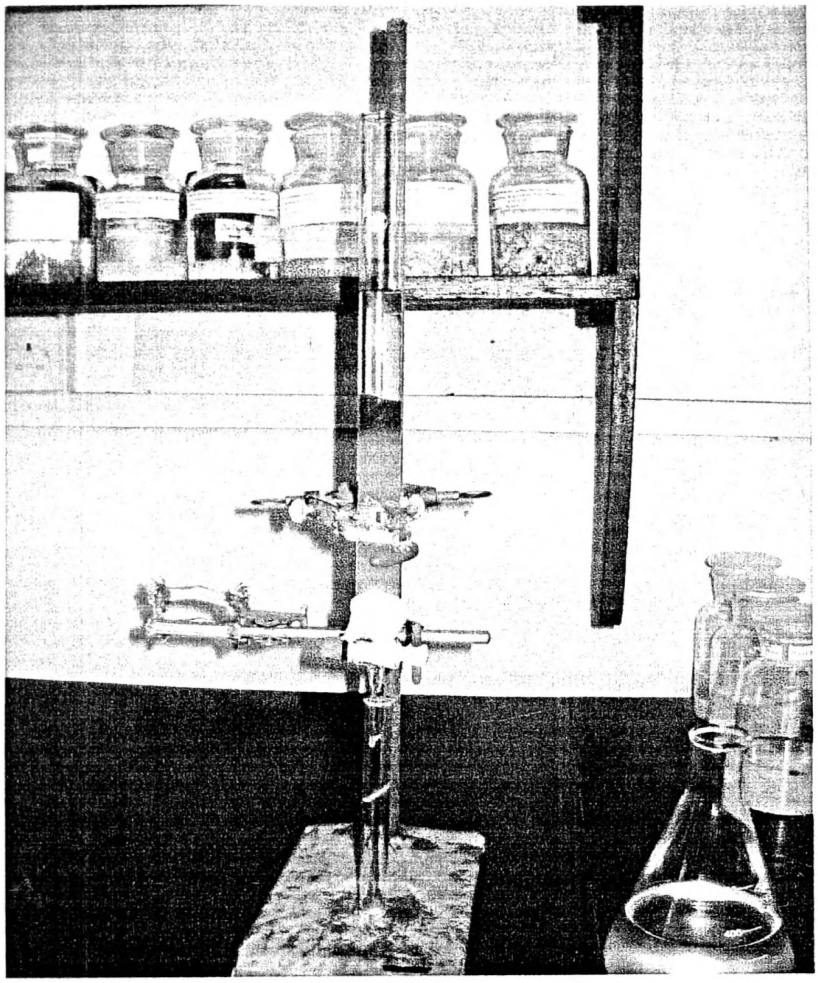

Fig. 3.1 Instrumentation of Column chromatography

145 fractions were collected from the main column and after analyzing each fraction using TLC, similar fractions were mixed. Then again analyzed the TLC plates of samples, one fraction having 45mg was rechromotographed using Hexane, EtOAc and MeOH by increasing the polarity and collected eight sub-fractions.

Again sub-fractions were analyzed by TLC and rechromotogrphed using C-18 silica gel instead of normal silica gel, with flash chromatography technique. By following this procedure several fractions of main column were divided in to sub-fractions. Finally five pure fractions were isolated.

3.2.5 Structure Elucidation of a compound isolated from green alga *Ulva lactuca* extract.

The structure was elucidated of a compound from *Ulva lactuca*[*] which observe as a yellow colored powder with melting point of 100 ^{0}C.

^{1}H NMR (300MHz, $CDCl_3$) δ; 5.27 (1H, m, H-9); 5.27 (1H, m, H-10); 2.28 (2H, t, H-2); 1.90 (2H, m, H-8); 1.90 (2H, m, H-11); 1.56 (2H, m, H-3); 1.3 (2H, bs, H-4); 1.3 (2H, bs, H-5); 1.3 (2H, bs, H-6); 1.3 (2H, bs, H-7); 1.3 (4H, bs, H-12); 1.3 (4H, bs, H-13); 1.3 (2H, bs, H-14); 1.3 (2H, bs, H-15); 1.3 (2H, bs, H-16); 1.3 (2H, bs, H-17); 1.2 (2H, bs, H-4); 1.2 (2H, bs, H-5); 1.2 (2H, bs, H-6); 1.2 (2H, bs, H-7); 1.2 (4H, bs, H-12); 1.2 (4H, bs, H-13); 1.2 (2H, bs, H-14); 1.2 (2H, bs, H-15); 0.84 (3H, t, H-18).

13C NMR (75MHz, CDCl3) δ; 179.91(C-1); 130.10(C-9); 130.00(C-10); 34.2(C-2); 32.10(C-16); 29.90(C-4); 29.90(C-5); 29.90(C-6); 29.90(C-7); 29.50(C-12); 29.50(C-13); 29.50(C-14); 29.50(C-15); 29.20(C-4); 29.20(C-12); 29.20(C-13); 29.20(C-14); 29.20(C-15); 27.50(C-11); 27.40(C-8); 24.90(C-3); 22.90(C-17); 14.30(C-18).

- Isolated by Mr. Haroon from Ulva lactuca At Institute of Fundamental studies, Kandy.

CHAPTER 4
RESULTS & DISCUSSION

Although a large portion of plant kingdom is acute by marine plants, compared with terrestrial plants, marine algae are still largely unexplored in chemically. The reasons for this may be difficulties in examine the ocean and difficult to reach and collect the marine algae. But presently studying about marine algae is vigorously increased, especially about chemical composition of them due to;

- Source of Natural Products which can exhibit biological activities such as antibiotic, antiviral, anticoagulant, antilipemic etc.
- One of the important daily food for Eastern people
- Usable as marine pollution indicator in environmental studies because they are endemic organisms and can thus reflect the pollution degree of the location where they grow up.

But according to the literature marine algae are rich source of biological active secondary metabolites. In recent studies of marine algae shows there are polar compounds appear in significant amount and they possess many biological activities. Ingredients of seaweeds are very important due to their involvement in human life in numerous ways; especially in pharmaceutical industry (Kamenatzka et al., 2006). Although now there is a new trend around the world to study and investigate the marine organisms, but in Sri Lanka still it is in poor stage.

So with the hope of exploring the seaweed world, around Sri Lanka, the institute of fundamental studies in Kandy started the Natural Product Program was started. Under this project Start to explore the brown alga *Padina pavonica,* Collected from Arugam Bay which is lay on eastern seashore of Sri Lanka, for the isolate chemical compounds possessing biological activity such as antifungal, antibacterial and cytotoxicity etc...All epiphytes were removed by washing with seawater and fresh water for several times. MeOH extract of algae were collected three times (3x 30min.) after sonicating the grinded plant material using an ultrasonicator.

MeOH was used to extract approximately all of the compounds in the plant material; because most of the compounds are dissolving in the methanol, so methanolic extraction of a plant material contain lots of compounds in several classes such as Fatty acids, Terpinoids, Sterols, amino acids and alkaloids etc...Without knowing the structure can't use any natural product for any clinical or pharmaceutical purposes. Chemical compounds of marine algae are exhibit in very small amount and they are very close in polarities. 3.0g of MeOH extract of *Padina pavonica* was obtained and it was subjected to column chromatography in silica gel columns with Hexane, Dichloromethane, Methanol and Distilled water as solvents. Elute was collected, as several fractions and after analyzing the TLC plates add similar fractions. But most of the TLC plates were very complex and had several compounds with close polarities in one fraction. With idea of getting pure compounds, Suitable fractions were rechromatographed with reducing the polarity gradient of solvents. In some cases, due to having a small amount of fraction and the polarities of them were close, use C-18 silica gel instead of normal silica gel. C-18 was used with water/Methanol solvent system, because they help to make good separation and they do not adsorb the compound. During the further purification used Hexane/Ethyl Acetate solvent system also. From the Hexane/ Dichloromethane fraction three pure compounds were isolated. One of them was yellow colored oil and other two were white colored powdered compounds. However, for the analyze of obtained pure compounds, the available instruments were not having sufficient sensitivity.

For the study and familiarization of spectroscopic techniques and structure elucidation, spectrums of compound (UL-4) which was obtained from *Ulva lactuca* was used.

4.1 Structure elucidation

The compound UL-4 was isolated from *Ulva lactuca* as a yellow colored, powdered compound. It was obtained by purify the fraction, which was get using 20% dichloromethane/hexane solvent system for the methanol extract of *Ulva lactuca* and show the melting point of 100 ^{0}C.

^{13}C NMR Spectrum (Fig. 4.2) of the UL-4 give the evidence for the presence of 18 carbon atoms in the molecular structure of it. Some carbon atoms from them were confirmed

with the use of literature (Forato, et al., 2004). By this spectrum showed a peak at δ_C 179.91 due to the presence of carbonyl carbon, two peaks appear at δ_C 130.1 and 131.0 and one methyle carbon at δ_C 14.3.

^{13}C DEPT spectrums (Fig. 4.3.1 and Fig. 4.3.2) indicate the presence of one methyle, 14 methylene, 2 methane and one quaternary carbon along the UL-4 structure.

Proton NMR spectrum (Fig.4.1) showed the presence of olefinic proton (CH=CH) resonance of C-9 and C-10 as multiplets at δ_H 5.27. Another proton resonance appears at δ_H 1.9 as multiplets, due to the allylic proton (C=CH-CH_2) of C-8 and C-11. A peak situated at δ_H 0.84 (J=6.9 Hz), describe for the proton resonance of C-18 as triplet. This spectrum showed the presence of methylene (CH_2) proton resonance multiplets at δ_H 1.2-1.3 in the alkyl chain. Proton NMR spectrum describe another peak at δ_H 2.28 (J=7.5 Hz) as a triplet, assign for the methylene proton resonance of C-2. Another methylene proton appeared at C-3 as multiplets at δ_H 1.56.

The connectivities among different groups were established and build the alkyl chain based on the HMBC correlation data. After examine the HMBC spectrum (Fig. 4.6) of UL-4 isolated from *Ulva lactuca*, the ^1H NMR signal as a triplet appears at δ_H 2.28 (J=7.5 Hz) showed correlation with carbons at δ_C 24.90 and 29.90 and the position δ_H 1.56 represented signal is correlated with carbons at δ_C 34.20 and 29.90. The high intensive peak at δ_H 1.2 showed correlation with ^{13}C NMR signals at δ_C 27.40 and 29.90 and another multiplet at δ_H 1.90 correlated with the carbon at δ_C 130.10 and also the carbons at δ_C 130.00 and 29.90. The signal appears at δ_H 5.27 showed correlation with the carbon appears at δ_C 27.40 and the signal appears at δ_H 1.3 correlated with the carbons at δ_C 32.40 and 14.30. The signal, which is having least chemical shift of ^1H NMR, appears at δ_H 0.84 (J=6.9 Hz) showed correlation with ^{13}C NMR signals appears at δ_C 22.90 and 32.10. After examine the HMQC spectrum (Fig. 4.4) of the compound isolated from the *Ulva lactuca*, Ul-4, give the clue for the chemical shifts of the all carbons attached to the protons (Tab. 4.1).

According to the HMBC and HMQC spectral data indicated that only one non-protonated carbon atom among the 18 carbons along the alkyl chain at δ_C 179.91 to be carbonyl. After analyzing the ^{13}C NMR spectrum there were two peaks around δ_C 130 and they were identified as an olefinic.

After examine and comparison with each one of the ^{1}H and ^{13}C NMR and other 2D spectral data proposed the molecular formula C18H34O2 and it was named as 9Z-octadecenoic acid or (Z)-Octadec-9-enoic acid (Oleic Acid).

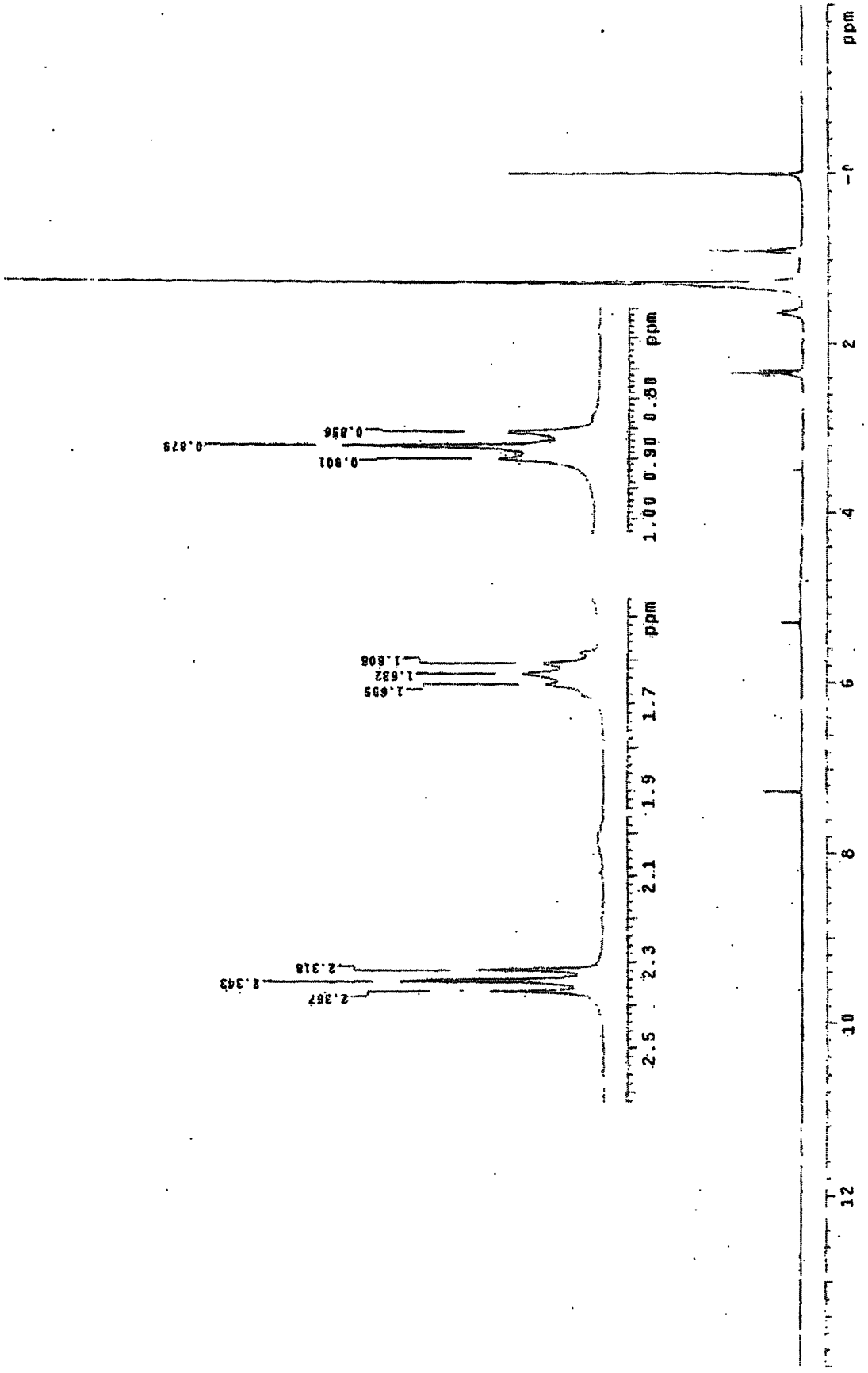

Fig. 4.1 ^{1}H NMR Spectrum

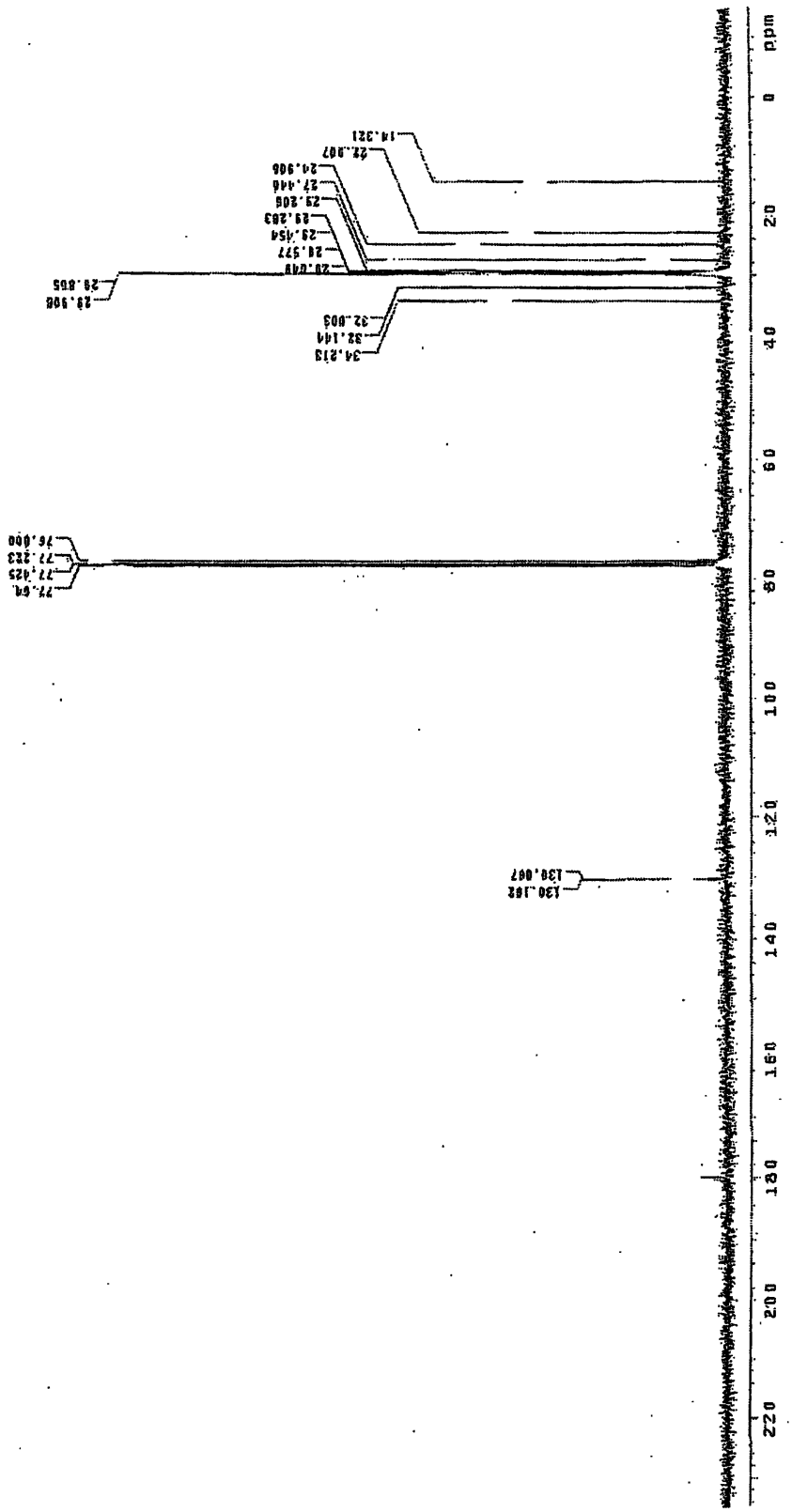

Fig. 4.2 ^{13}C NMR Spectrum

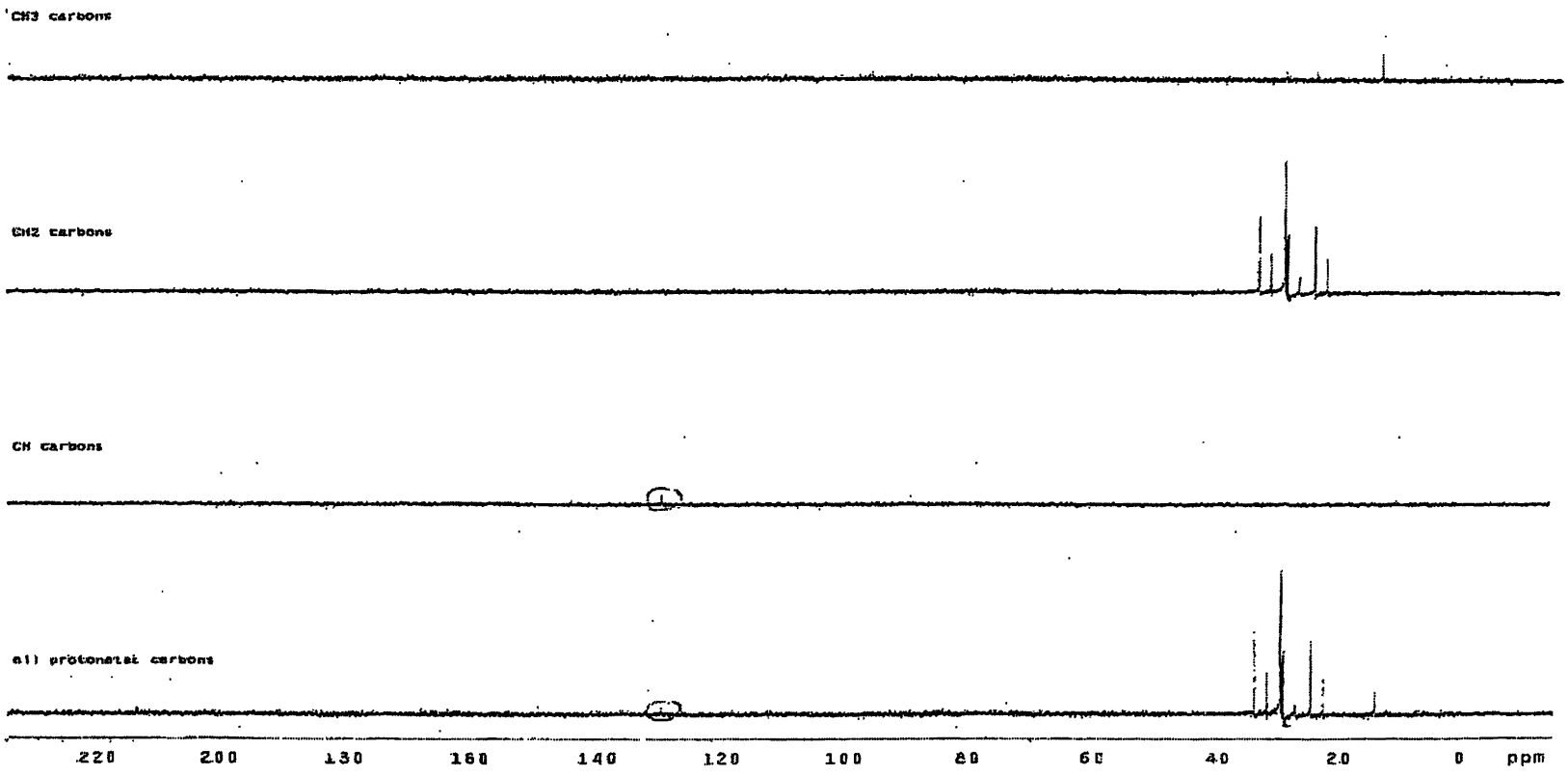

Fig. 4.3.1 ^{13}C DEPT Spectrum 1

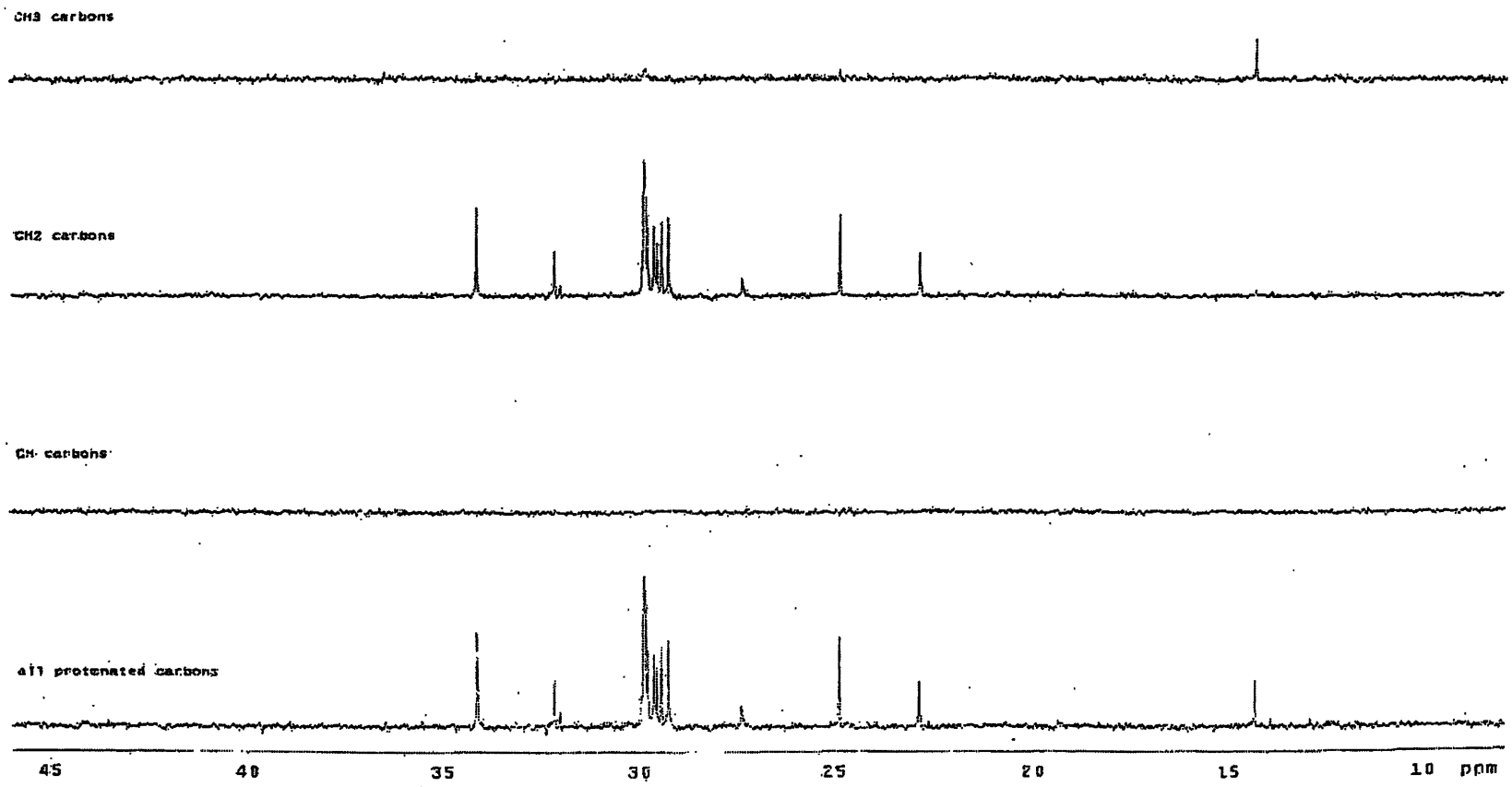

Fig. 4.3.2 ^{13}C DEPT Spectrum 2

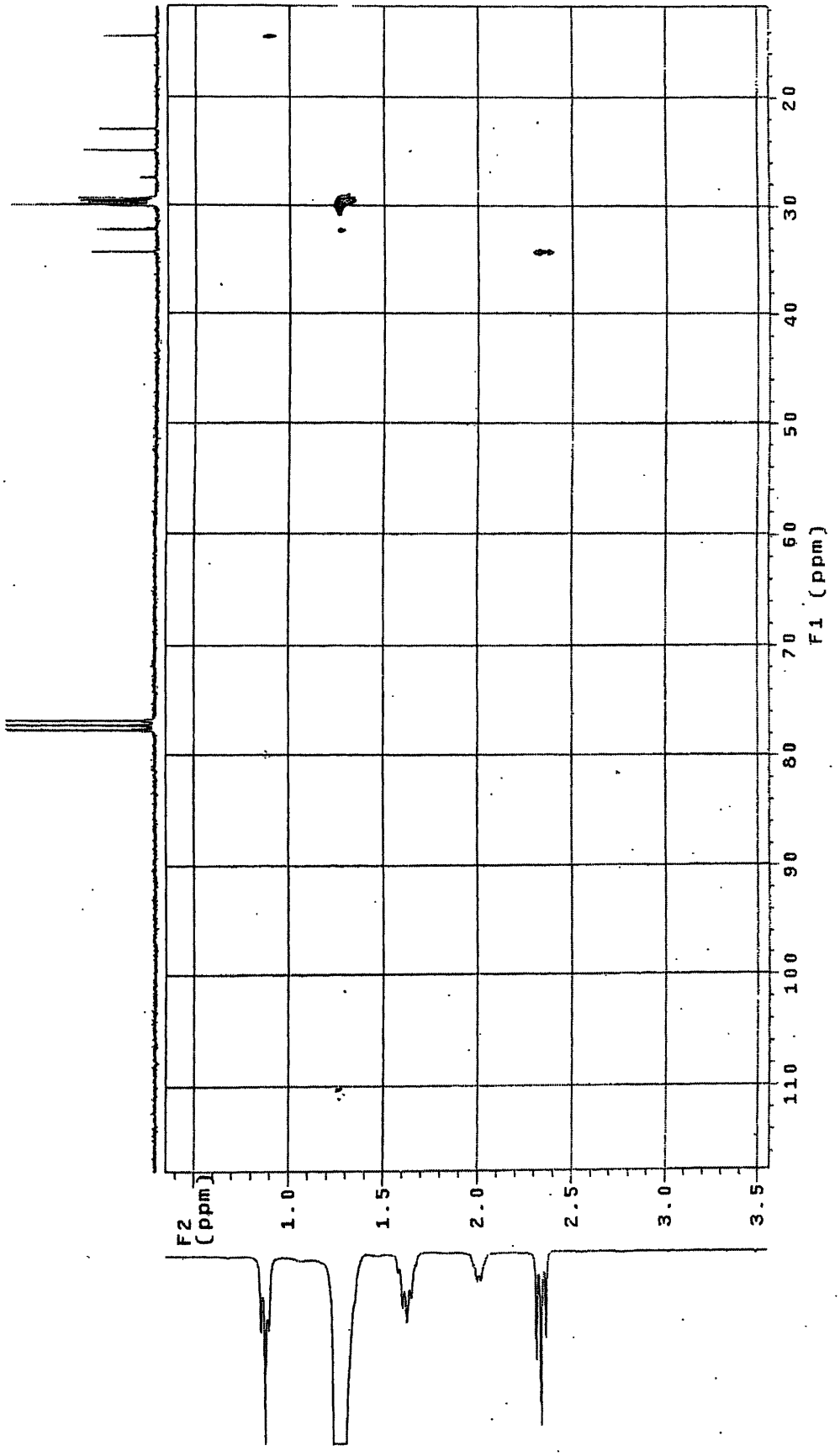

Fig. 4.4 HMQC Spectrum

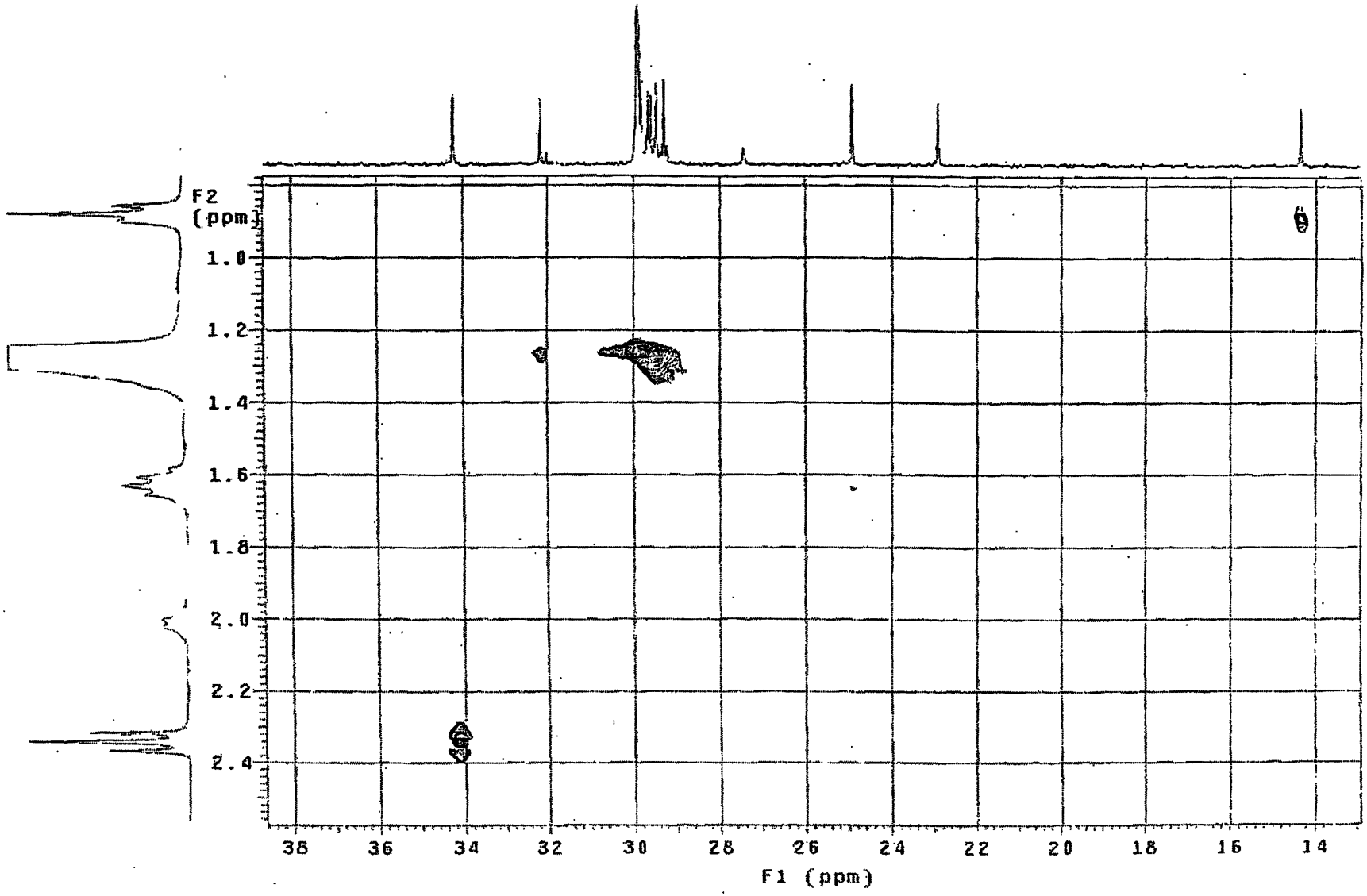

Fig. 4.5.1 Correlation Spectroscopy (COSY) 1

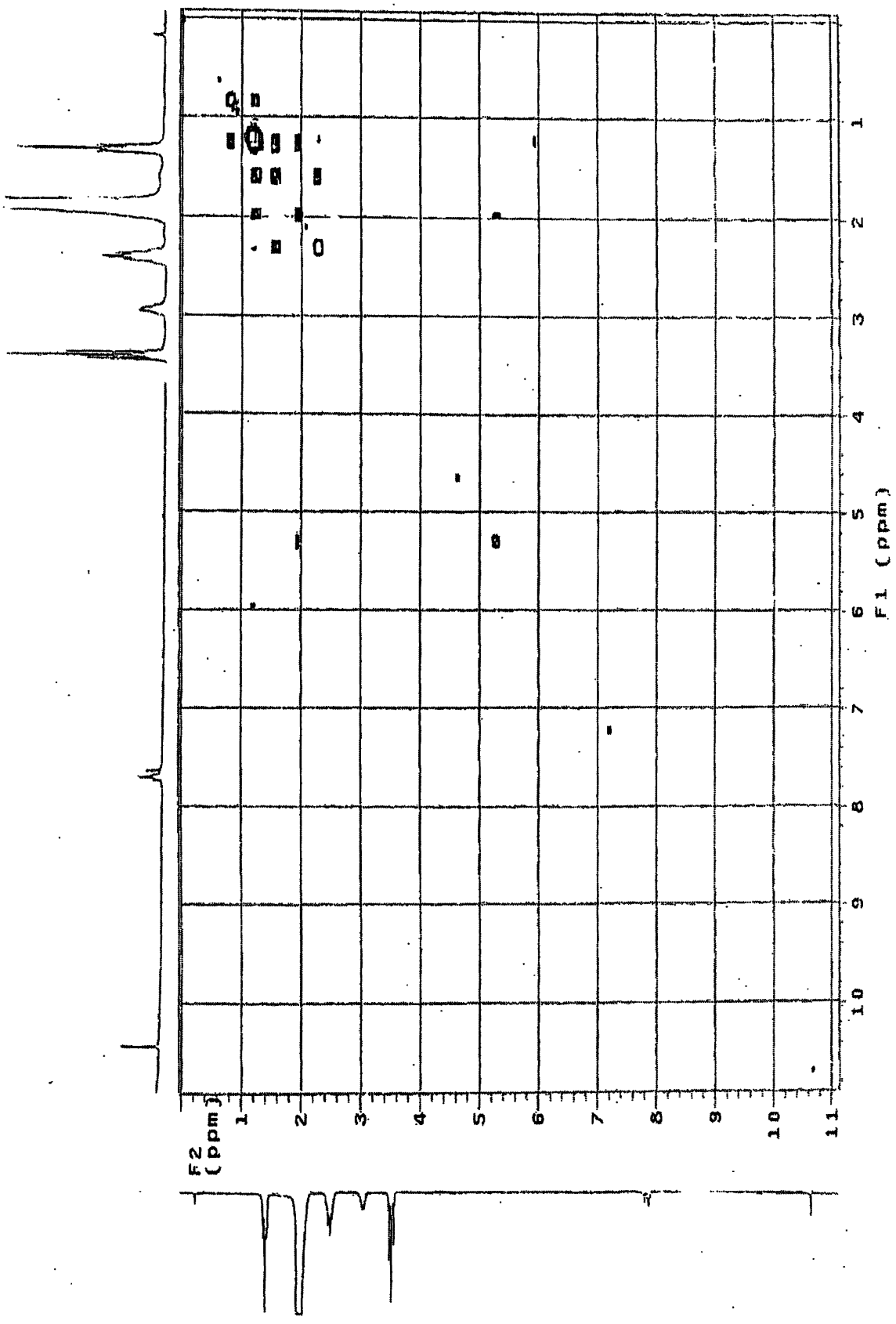

Fig. 4.5.2 Correlation Spectroscopy (COSY) 2

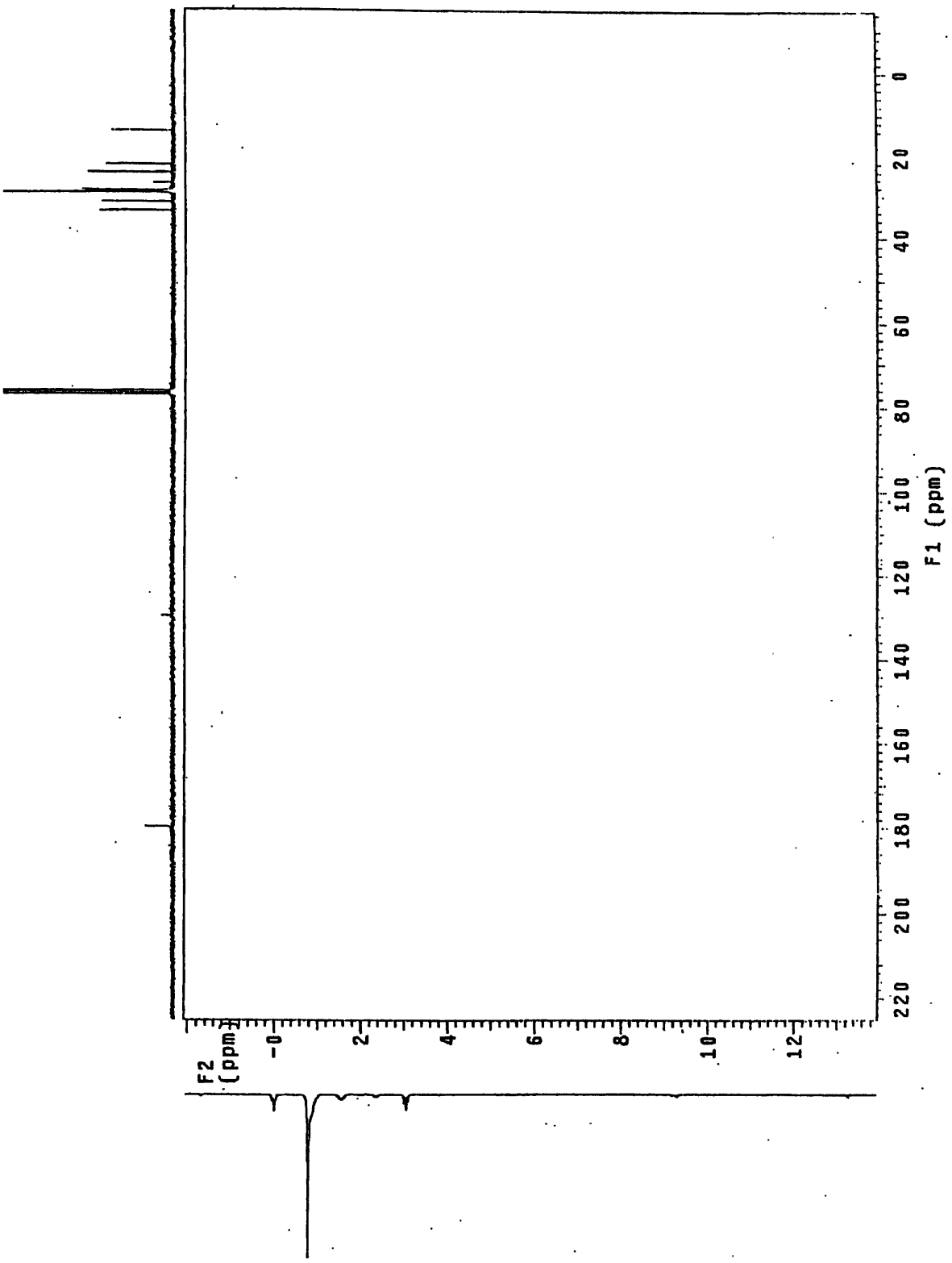

Fig. 4.6 HMBC Spectrum

Tab. 4.1 ^{1}H, ^{13}C NMR and HMBC data of compound UL-4 [300MHz, δppm, (*J*) Hz, $CDCl_3$]

Position of Carbon	Type of Carbon	^{1}H NMR (δ)	^{13}C NMR (δ)	HMBC correlations	
				HMBC - ^{1}H - ^{13}C (δ)	H/C Assignment
1	CO	-	179.91		C-2, C-3
2	CH_2	2.28(2H,t,7.5)	34.2	179.9, 24.9,29.90	C-3, C-4
3	CH_2	1.56 (2H,m)	24.9	179.9, 34.2, 29.90	C-2, C-4
4	CH_2	1.2-1.3 (2H,bs)	29.2-29.90	29.9, 24.9	C-3
5	CH_2	1.2-1.3 (2H, bs)	29.90	29.0	C-4
6	CH_2	1.2-1.3 (2H, bs)	29.90	27.40, 29.90	C-5
7	CH_2	1.2-1.3 (2H, bs)	29.9	27.40, 29.90	C-8
8	CH_2	1.90 (2H, m)	27.40	130.1, 130.0, 29.9	C-7, C-9
9	CH	5.27 (H,m)	130.1	27.40	C-8
10	CH	5.27 (H,m)	130.0	27.50	C-11
11	C=C-$\underline{CH_2}$	1.90 (2H,m)	27.50	130.1, 130.0, 29.0	C-12, C-10
12	CH_2	1.2-1.3 (4H,bs)	29.2-29.5	27.50, 29.4	C-11, C-13
13	CH_2	1.2-1.3 (4H,bs)	29.2-29.5	29.2-29.5	
14	CH_2	1.2-13 (2H,bs)	29.2-29.5	29.2-29.5	
15	CH_2	1.2-1.3 (2H, bs)	29.2-29.5		
16	CH_2	1.3 (2H, bs)	32.1	22.9,14.3,29.50	C-15, C17, C18
17	CH_2	1.3 (2H bs)	22.9	32.1, 14.3	C-16, C18
18	CH_3	0.84 (3H,t ,6.9)	14.3	22.9, 32.1	C-17, C 16

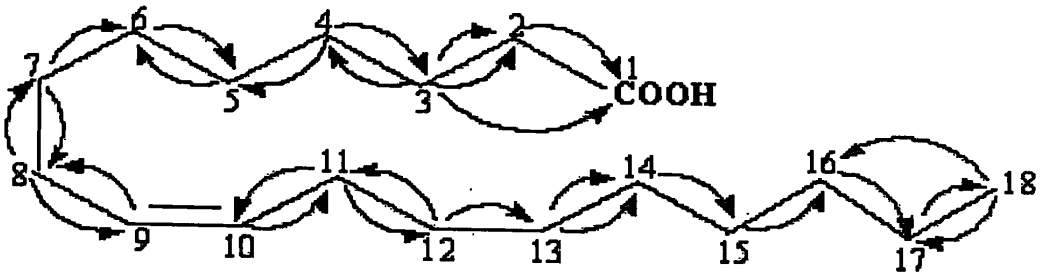

Fig. 4.7 HMBC correlation of UL-3

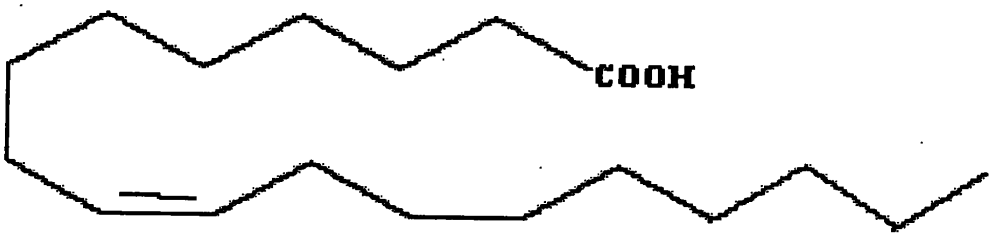

Fig. 4.8 9-octadecenoic acid (Oleic Acid)

CHAPTER 5
CONCLUSION & RECOMONDATION

5.1 Conclusion

Chemical investigation of the methanol extract of *Padina pavonica* was initiated with the hope of isolating compounds with biological activities such as antibacterial, antifungal, antioxidant, allelopathic, antitumor etc.... As we had very little amount of the Extract (3.0 g), I was not in a position to conduct a complete study on this seaweed. Therefore, at least 2Kg of the dry plant material (*Padina pavonica*) should be collected to carry out a complete study on Chemistry and biological activity of *Padina pavonica.*

^{1}H NMR spectrum of the compound UL-4, and its' streaking nature on TLC plate, indicated it to be a Fatty acid contaning olefinic carbon and the ^{13}C spectrum apparently showed 18 carbon atoms. After careful analysis of the DEPT, HMQC and HMBC spectra of UL-4, its' structure was proposed as 9-octadecenoic acid (Oleic Acid) having the chemical formulae of $C_{18}H_{34}O_2$. *Padina pavonica*

5.2 Recommendation

- To isolate more compounds from the MeOH extract of *Padina pavonica* need to use high performance separation techniques such as HPLC.
- The biological activities of the pure compounds isolated from the *Padina pavonica* need to be checked.
- The chemical composition of the *Padina pavonica* and the biological activity of the compounds of it can compare with other species of brown algae to check the importance of this alga.
- If get separate extractions for number of different solvents, the complexicity of the fractions can reduce and the purification process of these fractions may easy.

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APPENDIX I

Tab. I.I Percent relative composition of the fatty acid content as referred to the total fatty acid content in *Padina pavonica* from Saronikos Gulf of Greece.

Fatty Acid	**Percent relative composition**
Myristic Acid	3.09
Palmitic Acid	5.84
Palmitoleic Acid	8.29
Stearic Acid	8.84
Oleic Acid	28.93
Linoleic Acid	5.68
Linolenic Acid	8.79

Tab. I.II Percent relative composition of the Sterol content as referred to the total Sterol content in *Padina pavonica* from Saronikos Gulf of Greece.

Sterol	**Percent relative composition**
Cholesterol	68.68
Campesterol	4.49
Stigmaserol	4.57
Fucosterol	19.73

Tab. I.III Concentrations of the trace elements determined in *Padina pavonica* from Saronicos Gulf of Greece. (μg/g of dry matter)

Element	Fe	Sr	Zn	Co	Rb	Cs	Sc	Sb	Eu	Cr	Th
μg/g	950	2500	65	0.54	14	0.49	0.33	0.21	0.05	11	0.16

APPENDIX II

Tab. II.I The assignments of the most common proton signals relevant to fatty compounds

Structure	Description	Shift Values[a]
—CH_2—	cyclopropane	(-0.3) - 0.6
—CH_2—	cyclopropene	0.6 (singlet)
—CH_3	terminal methyl in alkyl chain	0.85-0.90 (triplet)
—CH_3	branched, saturated isoprenoid	0.85-0.90 (singlet or doublet)
—$C(CH_3)_2$	isopropyl methyl	1.2-1.3
(ω1)CH_2	saturated alkyl chain	1.2-1.3
—CH_2—	acyl C-3, saturated chains	1.58
—CH_2—	acyl C-4 to C-(ω3). saturated chains; (ω2)CH_2, saturated chain	1.2-1.3
RSH	sulfhydryl	1.1-1.5[b]
RNH_2	amino	1.1-1.5 (1.8)[b]
R_2NH	imino	0.4-1.6 (2.2)[b]
R_3C-H	saturated	1.4-1.7
—C≡C—CH_3	allylic methyl	1.6-1.9 (doublet)
—C≡C—CH_2—	allylic methylene	2.04 (doublet)
—C≡C—CH_2—C≡C—	diallylic methylene	2.8 (triplet)
—CH_2—COOR	acyl C2	2.1-2.3 (triplet)
—CH_2—CO—	α-methylene in ketone	2.2-2.5
COOR—CH_3	methyl in acetoxy	1.9-2.6 (singlet)
Ar—CH_3		2.1-2.5
—C≡C—H	terminal acetylene, non-conjugated	2.5-2.7
—O—CH_3	methoxy ether, aliphatic	3.3-3.8 (singlet)
—O—CH_3	methyl ester, aliphatic	3.6-3.8 (singlet)
—CH—OH	*sn*-2 in glycerol	3.75 (multiplet)
—CH_2—OH	*sn*-1 or *sn*-3 in glycerol	3.6 (doublet)
—O—CH_2—	aliphatic saturated alcohol or ether	3.4-3.7

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