2.0. REVIEW OF LITERATURE

2.1. MEDICINAL IMPORTANCE OF SEAWEEDS

Seaweeds were considered to be of medicinal value in the Orient as early as 3,000 B.C. The Chinese and Japanese used them in the treatment of goiter and other glandular diseases. Although the Romans believed seaweeds to be useless, they also used them to heal wounds, burns scurvy and rashes. The British used Porphyra to prevent scurvy during long voyages. Various red algae (particularly Coralline officinalis, C. rubens and Alsidium helminthocorton) were employed as vermifuges in ancient times. Several red algae (including Chondrus crispus, Gracilaria, Gelandium and Pterocladiia) have been used to treat various stomach and intestinal disorders.

The algae apparently absorb enough water and its water content helps relieving constipation and other associated discomforts. The stipes of Laminaria cloustoni have been used to aid in child birth by distending the uterus during labour. A number of specie of marine algae has been found to have anticoagulant and antibiotic properties. Carrageenan may be useful in ulcer therapy and the alginates are found to prolong the “rate of activity” of certain drugs (Mathieson, 1969). Species of Sargassum were used for cooling and blood cleaning effect. Hypnea musciformis was employed as vermifuge or worm expelling agent and Centroceras clavulatum as cathartic agent. The iodine rich seaweeds such as Asparagopsis taxiformis and Sarconema can be used for controlling goiter disease caused by the enlargement of thyroid gland (Umamaheswara Rao, 1970).
Though the importance of different seaweed products in pharmacology is known, the development of antimicrobial, antifungal and antiviral substances from seaweeds is still in an initial stage of research and development. Extracts from Chondrus crispus and Gelidium cartilagineum have been found to be active against influenza-B and mumps virus, (Garber et al., 1958). Henriquez et al., (1979) assayed 33 species of Chilean marine algae for their antibacterial activity against Sarcina lutea ATCC 001, Staphylococcus aureus ATCC 65388 and Bacillus subtilis ATCC 6633. Some degree of antibacterial activity was found to be present in 17 of these 33 extracts. Caccamese et al., (1980) tested lipid extracts of more than 20 algae from eastern Sicily for antimicrobial activity against tobacco mosaic virus. Some of them mainly belonging to Dictyotales were found to be bioactive. Caccamese et al., (1981) tested lipid extracts of 13 algae from Eastern Sicily for antimicrobial activity against Bacillus subtilis and Phoma tracheiphila and for antiviral activity against tobacco mosaic virus. Zanardine prototypes and Cystoseira balearica exhibited the best antimicrobial and antiviral activity among the species tested. Blunden et al., (1981) examined the extract of British marine algae for anti-influenza virus activity based on inhibition of influenza neuraminidase.

The antibacterial and antifungal activity of Indian seaweed extracts (Sreenivasa Rao et al., 1979c; Sresnivasa Rao and Shelat, 1979 and Sreenivasa Rao and Parekh, 1981) and also the effect of seaweed extracts on Mycobacterium tuberculosis (Sreenivasa Rao et al., 1979d) have been studied. Antibiotic substance isolated from Enteromorpha effected complete inhibition of growth of the tubercle bacilli in the culture. Naqvi et al., (1981) examined the extracts of 25 seaweeds from Indian coast
for antiviral, antibacterial antifungal, antiprotozoal, antifertility activities. Significant biological activity was obtained in 13 species of seaweeds, the most promising activity being 100% antifertility (anti-implantation) activity observed in 3 species namely *Padina tetrastromatica, Gelidiella acerosa* and *Acanthophora spicifera*.

Seaweed has been a source for the production of a variety of major metabolites such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics and many other fine chemicals (Targett & Mitsui, 1979; Fenical, 1982; Colwell, 1983; Stein & Borden, 1984). Algal dietary fibre, ranged from 33 % to 75 % of dry weight, mainly consists of soluble polysaccharides having important functional activities such as antioxidant, anticoagulant, antimutagenic and antitumor activities and have an important role in the modification of lipid metabolism in human body (Effimov *et al.*, 1983; Guvon *et al.*, 1973; Ito & Sugiura, 1976; Okai *et al.*, 1996). Gelatinous preparation of the seaweed *Ceramium loreirii* is used for chest disease in coastal areas of the pacific (Hoppe, 1979).

Seaweeds have curative powers for tuberculosis, arthritis, colds and influenza, and worm infections. Dry *Laminaria* stripes have long been used in obstetrics to dilate the cervix and were known as “*Laminaria tents*” (Stein & Border, 1984). Seaweeds are not only the source of major metabolites but are an extensive prolific source of secondary metabolites, More than 600 secondary metabolites have been isolated from marine algae (Faulkner, 1984 and 1986). Many of these compounds are bioactive and have been extensively studied using bioassays and pharmacological assays (Paul & Fenical, 1987). Antioxidant property has been studied in the edible brown, green and red seaweeds in which the antioxidative property has been correlated to their phenolic
content (Jimenez-Escrig et al., 2001). Marine plants, like seaweeds, also contain high amounts of polyphenols. For example, high concentrations of polyphenols such as catechin, epicatechin, epigallocatechin gallate and gallic acid are reported in the seaweed Halimada (Chlorophyceae) (Yoshie et al., 2002). The sulphated polysaccharides of Sargassum have been reported to act as a potent free radical scavenger and anticancer agent (Park et al., 2005; Dias et al., 2005). The methanolic extract of brown seaweeds such as Hizikia fusiformis Siriwardhana (2003) and Ecklonia cava Senevirathne (2006) have been reported to exhibit potent antioxidant activity. Since many types of seaweed have still to be investigated, which prompted to take up the present research study.

2.2. PHYTOCHEMICAL ANALYSIS OF SEAWEEDS

Studies have been made on the chemical and proximate composition of seaweed mainly by Chidambaram and Unny (1947) and Joseph and Mahadevan (1948). Mehta and Baxi (1976) studied the mineral constituents and ion-exchange property of some brown seaweed from the Indian coast. Parekh et al. (1977) estimated the chemical composition of green algae of Saurashtra coast. Lipid, sterol and chlorophyll contents of some of the Indian seaweeds were studied by Parekh et al. (1983). Dave and Chauhan (1985) estimated the protein content of brown seaweeds from Gujarat coast. Chennubhotla et al. (1987b) reported that the food value of the seaweeds depend largely on their mineral, trace element, protein and vitamin content. Minerals, carbohydrate and other chemical constituents of seaweeds were estimated by Kaliaperumal et al. (1987). Like mineral constituents, lipid content of seaweed was also studied by Parekh and Chauhan (1987). Kesava Rao and Indusekhar (1987)
described the carbon, nitrogen and phosphorous ratios in the seaweeds of Saurashtra coast. Protein content of red seaweeds of Gujarat coast was estimated by Dave et al. (1987). Biochemical composition of the marine algae from Mandapam coast and Tuticorin coast, Gulf of Mannar region has been studied by many workers (Chennubhotla et al., 1987b; Reetha Jayasankar et al., 1990; Venkataraman Kumar, 1993a, 1993b, 2005; Ganesan and Kannan, 1994; Kaliaperumal et al., 2002; Vimalabai et al., 2003).

The biochemical constituents of Gracilaria edulis cultured from spores were studied by Reetha Jayasankar et al., (2005). Seasonal variation in biochemical constituents of Grateloupia lithophila were reported by Rajasulochana et al., (2005). Devi et al., (2008) and Rajalakshmi et al., (2008) have carried out the phytochemical analysis of seaweeds from Tuticorin Coast, and Mallipattinam Coast, Tamil Nadu respectively. The brown alga Sargassum tenerrium collected from Mandapam was analysed for biochemical composition of protein, lipid, carbohydrate, amino acids and fatty acids (Gandhiyappa et al., 2008). Study was made for macro and micro nutrients in some selected economically important seaweeds occurring in the Southeast Coast of Tamil Nadu (Naganatham et al., 2008). Protein content of some marine macro alga form Katumavadi Coast, Tamil Nadu was determined by Arumugam et al., 2009. The mineral composition of some green seaweeds of Vishakapatnam Coast has been studied by Sarojini and Lakshminarayanan, 2009. Biochemical analysis of 15 seaweeds belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae from Chidiyatapu, South Andaman was carried out Palanisamy (2010). Murugaiyan and Sivakumar (2010) have studied the seasonal variation in chemical composition of
certain seaweeds from Pudumandapam, Southeast Coast of Tamil Nadu. The biochemical composition of seaweeds of Mandapam Coast has been studied by Meenakshi et al., 2010.

2.3. ANTIDIABETIC PROPERTY (ANTIOXIDANT & HYPOGLYCAEMIC ACTIVITY)

Today, more than 1000 plants have been identified as potential treatment for diabetes but only a few of them have been evaluated scientifically to determine their effectiveness (Bailey and Day, 1989). For some plants, hypoglycemic effect has been demonstrated in different animal models but those active compounds have not been isolated and identified. In some other plants that are considered potential hypoglycaemic agents have been determined without clinical evaluation (Ivorra et al., 1989). Newer oral hypoglycaemic agents (OHA) and various insulin preparations have been developed in the recent past. Many OHA sulphonylurea compounds (e.g., Tolbutamide, Acetohexamide, Chloropropamide, Glybenclamide, Glipizide and Glitazide) which exert their hypoglycaemic effect, mainly, by increasing insulin secretion as well as potentiation of insulin action (Kahn and Shechter, 1990).

Another class of OHA is guanidine derivatives known as biguanides (e.g. phenformin, metformin and biuformin), which produce insulin like effects on several tissues and do not cause hypoglycaemia in normal subjects. However, limitations of OHA in NIDDM and problems of insulin antibodies in IDDM have been recorded (Kahn and Shechter, 1990). Although insulin seems an ideal antidiabetic agent, it cannot stimulate the pancreas of a healthy individual. Hence diabetics within 15 to 20 years of diagnosis begin to develop complications (Brownlee and Cerami, 1981). The
range of different complications in diabetes is directly related to the hour-to-hour fluctuations in blood sugar that persist even when insulin is taken once or twice a day (Job et al., 1975; Pirart, 1978). Diabetes is the leading case of non-congenial cardiovascular problem and kidney failure.

Extracts of marine plants have been reported to be potentially useful hypoglycaemic agents in the treatment of diabetes. Recently it has been reported that the active hypoglycaemic principle of the bark is (-) epicatechin (Sheehan and Zemaitis, 1983). Furthermore it has been reported that the hypoglycaemic effect of this compound is due to regeneration of beta cells in the pancreatic islets of alloxan diabetic rats (Hii and Howell, 1984; Chakravarthy et al., 1981). Epidemiological data also warn that diabetic patients are still at risk of chronic morbidity and premature mortality even after the assiduous use of the available OHA. A normal physiological pattern of glucose homeostasis is rarely reinstated after treatment (Aberti and Zimmet, 1998). The pectin isolated from the juice of the seaweeds has been found to show hypoglycaemic effects both in normoglycaemic and alloxan induced diabetic rats. A dose of 20 mg increased the concentration of hepatic glycogen and glycogenesis as evidenced from the increased activity of glycogen synthetase and increased incorporation of labelled glucose into hepatic glycogen in normoglycaemic rats (Gomathy et al., 1990).

Diabetic patients thus have an increased incidence of vascular diseases and it has been suggested that free radical activity increased in diabetes (Oberley, 1988). It has also been shown that glucose under physiological conditions produces oxidant that possesses reactivity similar to the hydroxyl-free radicals. Recent years have witnessed
a renewed interest in marine plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS) (Cao et al., 1997; Vaya et al., 1997). Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of the medicinal plant from marine environment for the treatments of diabetes have received scientific scrutiny, for which WHO has also recommended attention (WHO, 1980).

Free radical-induced LPO has been associated with a number of disease processes including diabetes mellitus (Feillet et al., 1999). The increase in oxygen-free radicals in diabetes could be due to increase in blood glucose levels, which generate free radicals upon autoxidation (Venkateswaran and Pari, 2003). Glucose auto-oxidises in the presence of transition metal ions generating oxygen-free radicals which make the membrane vulnerable to oxidative damage. The action of diabetes-inducing agents produces reactive free radicals, which have been shown to be cytotoxic to the β-cells of the pancreas (Heikkila et al., 1976). As on date there is no detailed investigation to determine the hypoglyceamic effect of brown seaweed S. wightii. So the present study has been undertaken to investigate the effect of S. wightii on blood glucose levels in glucose fed hyperglycaemic, alloxan induced diabetic and normal rats.

2.4. ANTIOBESITIC PROPERTY

Seaweeds have been used as drug or drug sources over a large number of years going back into folk medicine. One of the pharmacologically interesting properties of certain marine algae is their hypocholesterolemic effect. Kaneda et al., (1963) studied
the hypocholesterolemic effects of several marine algae in cholesterol-fed rats. The daily addition of “sea products” (such as seaweed and fish oils) to diet for prophylaxis of coronary atherosclerosis has been suggested (Chunakova, 1967; Velichko & Shevchenko, 1998). In 1969, Tsuchiya reported the hypocholesterolemic activities of a number of marine algae Enteromorpha eumprura, E. prolifera, Ulva pertusa, Monostroma indium, six species of Laminaria, Costaria costata, Sargassum muticum, Fucus gardneri, two species of Kjellmaniella, Athrothamnus bifidus, Undaria (two species), Heterochordaria abietina, Chondrus and Porphyra tenera. The responsible compounds are sterols such as ergosterols, chondriosterol, poriferasterol and other for the depression of blood pressure in human atherosclerosis. Similarly Laminaria species which are traditionally used for the treatment of goiter in folk medicine also lowered blood pressure and cholesterol level (Hoppe, 1979). Red seaweed species containing non-toxic sterols have been reported to reduce blood cholesterol level and retard the accumulation of fat in liver and heart (Bhakuni & Silva, 1974; Patterson, 1977). Unsaturated fatty acids isolated from Heterochordoria abietina were capable of depressing the blood cholesterol level in rats (Marderosian, 1970). The effect of laminarian sulfate on serum lipid profile of experimental atherosclerotic rabbits subjected to long-term intermittent cholesterol feeding. Laminarian sulphate extracted from brown seaweed, exhibited antilipidaemic activity (Michanek, 1979; Tsuchiya, 1969; Wang & Yang, 1997).

The dietary and pharmacological lowering of elevated plasma LDL cholesterol appears to be one of the methods to reduce the development of atherosclerosis. Over the past several decades, tremendous efforts have been underway to develop effective
therapeutic hypolipidaemic agents, which reduce VLDL and LDL cholesterol, increase HDL cholesterol and reduce triglyceride levels without significant side effects. This had led to the discovery of hundreds of compounds that showed significant serum cholesterol and triglyceride lowering effects, such as bile acid sequestrates, nicotinic acid, fibrin acids, 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitors, probucol and many others (Marx, 1979).

Several polysaccharides from red and brown algae have also shown hypocholesterolaemic activity (Bhakuni & Silva, 1974; Guven et al., 1979; Vazquez-Freire et al., 1996). Besteman (1970) reported hypolipidaemic effect of laminarian sulphate in rabbits. Similarly Michanek (1979) demonstrated the hypocholesterolaemic effect of carrageenan, agar and alginic acid. Due to the positive association between plasma cholesterol, LDL-cholesterol and the incidence of the heart diseases, national health organizations in several countries have stressed the importance of reducing plasma cholesterol and LDL-cholesterol levels for prevention of coronary heart diseases. The current study is therefore aimed towards the utilization of marine macro-algal species S. wightii for pharmaceutical benefits and to examine the hypolipidaemic activity of this seaweed occurring at Hare Island.

2.5. CARDIOPROTECTIVE PROPERTY

Coronary heart disease is the most common form of heart disease and the single most important cause of premature death in the developed world. The risk factors for coronary heart disease are based on the extensive data demonstrating the relationship between exposure to causative environmental factors and occurrence of clinical illness are smoking, hypertension, hypercholesterolemia, diabetes mellitus,
haemostatic factors, obesity, alcohol, other dietary factors, and mental stress (Boon et al., 1999). Ischemia is recognized when it is sufficient to cause characteristic metabolic and ECG changes that are reversible when the ischemia ceases. Ischemia is therefore, by definition, a reversible deficiency of myocardial blood supply. As ischemia progresses, ultimately a state of infarction develops (Berhard et al., 1991).

Myocardial oxygen supply may therefore be influenced by alterations in vasomotor tone, with ischemic episodes resulting from vasoconstriction of such normal segments of stenosis. Episodes of ischemia may also result from platelet aggregation at the sites of coronary stenosis resulting in transient obstruction to coronary blood flow and reduced oxygen supply (Anonymous, 1999). Myocardial infarction follows the recent occlusion of the two major coronary arteries (Right and left coronary arteries) or it results from the extreme narrowing of one or more large branches (Nissler and Vesselinovitch 1968). The infarction lies within the area of myocardium supplied by the recently occluded vessel. Myocardial infarction is continued almost exclusively to some portion of the left ventricle or the interventricular septum of the right ventricle is rarely involved. Occlusion of left anterior descending coronary artery (LAD) causes infarction of the anterior wall of the left ventricle near the apex (Murray et al., 1985). Right coronary artery occlusion leads to infarction in the interior and posterior left ventricle. Myocardial infarction of left ventricle is cause by the occlusions of the left main coronary artery. Thrombosis seems to be one of the main process leading to active myocardial infarction. Marine macro algae or the so called seaweeds have been found to possess anticoagulant and antithrombotic agents. These agents have been characterized as anticoagulant and
antithrombotic polysaccharides. These polysaccharides constitute potential therapeutic compounds for treating thrombosis without any side effects (Mourao, 2004). Literature on cardioprotective agents especially from marine plants like the seaweeds is very meager, so the present study was taken to assess the cardioprotective property of brown seaweed *S. wightii*. 