1.0. GENERAL INTRODUCTION

There are lot of life threatening diseases have identified in human beings. Many studies have shown that free radicals and their derivatives, which are produced during metabolism or environmental impact, could induce the damage of biomolecules and promote life-threatening health problems, such as aging (Mau et al., 2002), arthritis (Biemond et al., 1984), cardiovascular diseases (Gey, 1993), cancer (Chihara et al., 1970), diabetes (Xue et al., 2009), inflammation (Carbonero et al., 2008), Parkinson’s diseases and other disorders. Among them, diabetes mellitus is a metabolic disorder characterized by hyperglycemia caused by insulin deficiency, which affects the metabolism of carbohydrates, fats and proteins (Sexton and Jarow, 1997; Rao et al., 2001). According to the International Diabetes Federation, India has been declared as “Diabetic capital of the world” at a conference in Paris (Bezbaruah, 2003). There are two main forms of diabetes namely type-1 and type-2. Among them, later is more prevalent and account for about 90 to 95% of all diagnosed cases of diabetes (Skyler, 2004). Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with autoimmune destruction of pancreatic β-cells, and this disease is more likely to occur in relatives of an affected person (Bottini et al., 2006). Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion (Warren, 2004).

Diabetes mellitus is a disease characterized by chronic hyperglycemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result in the development of further metabolic and anatomic disturbances like
lipemia, hypercholesterolemia, loss of weight, ketosis, arteriosclerosis, renal disease and coma (Andrew et al., 2000). Hyperglycemia and glucose intolerance are common manifestations of several types of hormonal disturbances or imbalances, of which the most important is diabetes mellitus (Forster, 1987). This disease is the seventh leading cause of death in the world. Weight loss, which is one of the clinical features of diabetes mellitus may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy loss from the body due to the frequent urination and over the conversion of glycogen to glucose. Weight loss is a serious issue in the management of diabetes mellitus (Reno and Leland, 1999).

Diabetes mellitus is associated with degenerative and functional disorders of the central nervous system such as the impairment of learning and memory, high risk of dementia, Alzheimer’s disease and depressions (Ott et al., 1997). The cardiovascular diseases constitute the main cause of morbidity and mortality in diabetes especially in type-2 diabetes. Glucocorticoids are important regulators of metabolic process, including the stimulation of gluconeogenesis and the regulation of fatty acid synthesis. Elevated glucocorticoids have been associated with visceral obesity, hyperglycemia, insulin resistance and diabetes type-2 (Antanasov and Odermatt, 2007). Patients with type-2 diabetes exhibit a marked reduction in insulin mediated glucose disposal (Reaven, 1983). This is because the ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose level (Kruszynska, 1996). Additionally insulin resistance is independently associated with obesity (Gao et al., 2007) and it is noted that insulin resistance is more severe in these patients (Seely and Olesky, 1993).
The control of diabetes mellitus normally involves exercise, diet and drug theory. Extensive research and development on diabetes leads to a number of synthetic oral hypoglycemic agents, but the disease and its related complications remain uncontrolled (Ritz and Orth, 1999). Moreover, most of the synthetic hypoglycemic agents are associated with serious adverse effects. On the other hand, traditional medicinal plants with their diverse phytoconstituents have been employed successfully by the communities from long time to treat diabetes without producing demonstrable adverse effects. In the last years, there has been an increasing demand for natural products with antidiabetic activity, mainly due to the side effects associated with the use of insulin and oral hypoglycemic agents. The search for a cure for diabetes mellitus continues along traditional and alternative medicines. Many herbal supplements have been used for the treatment of diabetes, but not all they have scientific evidence to support their effectiveness (Morelli and Roger, 2000).

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylurease, biguanides, alpha glucosidase inhibitors, glinides and biguanides (Dalziel, 1995; Li and Yin, 2005), but these synthetic agents are associated with drawbacks such as rigid and multiple dosing regimen, high-cost and inaccessibility (De Melo et al., 2002). Therefore the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Despite, the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have found to be useful to successfully manage diabetes. One of the greatest advantages of traditional
medicinal plants is that these are readily available and have no side effects (Mehta, 1982). Many researchers have studied more than twenty medicinal plants, such as American ginseng berry (Xie et al., 2004), green tea (Chen et al., 2005), Astragalus membranaceus (Zhou et al., 2005; Guo et al., 2007), Cordyceps militaris and C. sinensis (Kim and Yun, 2005), etc., acquiring that polysaccharides from these medicinal plants have hypoglycemic effects. As a result, more and more people pay close attention to plant polysaccharides. Plants have been an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. It is reported that about 800 plants may possess anti-diabetic potential (Grover et al., 2002).

All living organisms contain complex systems of antioxidant enzymes. Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signalling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species. Reactive oxygen species include hydrogen peroxide (H₂O₂), the superoxide anion (O²⁻), and free radicals, such as the hydroxyl radical (·OH). These molecules are unstable and highly reactive, and can damage cells by chain reactions, such as lipid peroxidation, or formation of DNA adducts that could cause cancer-promoting mutations or cell death. In order to reduce or prevent this damage, all cells invariably contain antioxidants.

Recently, the oxidation of lipids has received renewed attention with increasing evidence showing that lipid peroxidation is one of the important primary
events in the free radical-mediated oxidative damage of biological membranes and tissues. Antioxidants are organic molecules which can prevent or delay the progress of lipid oxidation. Their ability to do this is based mainly on their phenol-derived structure. Lately, the interest in using antioxidants of natural origin in food has increased, because they also appear to be suitable antioxidants for the prevention of diseases associated with the process of lipid peroxidation (Gordon, 1996; Valenzuela et al., 2003).

Lipid oxidation by reactive oxygen species (ROS), such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, causes a decrease in nutritional value of lipids, in their safety and appearance. In addition, it is the predominant cause of qualitative decay of foods, which leads to rancidity, toxicity, and destruction of biochemical components important in physiological metabolism. Free radical mediated modification of DNA, proteins, lipids and small cellular molecules are associated with a number of pathological processes, including atherosclerosis, arthritis, diabetes, cataractogenesis, muscular dystrophy, pulmonary dysfunction, inflammatory disorders, ischemiareperfusion tissue damage, and neurological disorders, such as Alzheimer’s disease (Frlich and Riederer, 1995). Antioxidants are classified by the products they form on oxidation (these can be antioxidants themselves, inert or pro-oxidant), by what happens to the oxidation products (the antioxidant may be regenerated by different antioxidants or, in the case of “sacrificial” antioxidants, its oxidized form may be broken down by the organism) and how effective the antioxidant is against specific free radicals. Several synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene
(BHT), and tert-butylhydroquinone (TBHQ) are commercially available and currently used. However, these antioxidants have been restricted for use in foods as they are suspected to be carcinogenic. Some toxicological studies have also implicated the use of these synthetic antioxidants in promoting the development of cancerous cells in rats. These findings, together with consumers’ interests in natural food additives, have reinforced the efforts for the development of alternative antioxidants of natural origin (Huang and Wang, 2004). An immense number of marine flora and fauna are reported to have a wide spectrum of interesting biological properties. In folk medicine, seaweeds have been used for a variety of remedial purposes, e.g. for the treatment of eczema, gallstone, gout, crofula, cooling agent for fever, menstrual trouble, renal problems and scabies (Chapman and Chapman, 1976).

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Despite considerable progress in medical research, cancer remains one of the high-ranking causes of death in the world. The National Cancer Institute estimates that “approximately 11.4 million Americans with a history of cancer were alive in January 2006. In 2012, about 577,190 Americans were died of cancer, more than 1500 people a day. Cancer is the second most common cause of death in the US, exceeded only by heart disease, accounting for nearly 1 of every 4 deaths (Source: Cancer Facts and Figures 2012 of the American Cancer Society).

Cancer is a leading cause of death worldwide and a diverse group of diseases characterized by the uncontrolled proliferation of anaplastic cells which tend to
invade surrounding tissues and metastasize to other tissues and organs. Cancer results from a mutation in the chromosomal DNA of a normal cell, which can be triggered by both external factors (tobacco, alcohol, chemicals, infectious agents and radiation) and internal factors (hormones, immune conditions, inherited mutations, and mutations occurring in metabolism). A report released by the World Health Organization (WHO) showed that an estimated 12.7 million people were diagnosed with cancer globally and about 7.6 million people died of it in 2008. As estimated in this report, more than 21 million new cancer cases and 13 million deaths are expected by 2030. Although cancer accounts for around 13% of all deaths in the world, more than 30% of cancer deaths can be prevented by modifying or avoiding key risk factors (WHO, 2011).

Accordingly, research must continue to progress to improve existing therapies for all the above said diseases and to develop novel cures. For many years, research has essentially focused on plants and terrestrial microorganisms, mainly because of these specimens are easily available and folk traditions have described beneficial effects from their use. Several different therapeutic strategies such as chemotherapy, radiation therapy, surgery or combination of the above have been used to treat different types of cancers. Unfortunately, several of these treatments provide only minimal benefits; moreover, complications and long term side effects may happened through these treatments (Grossi et al., 2010; Schneider et al., 2010).

Wound healing is a dynamic process that combines tissue-regeneration events with local activation of immune functions. To defeat infection, wound
treatment involves diverse cell types—(i) keratinocytes, which regenerate the cutaneous barrier, (ii) dermal fibroblasts, which produce an extracellular-matrix scaffold and (iii) monocytes, which chemotactically infiltrate the wound and produce cytokines that stimulate different functions, including immunity (Massague, 1999). The principal functions of wound dressings are to remove wound exudates, to prevent the entry of harmful bacteria into the wound, and to promote the establishment of the best milieu for natural healing (Boateng et al., 2008; Ovington, 2007).

Fibres have been extensively used in wound dressing applications, because of their unique/advantageous properties, such as high surface area, softness, absorbency and ease of fabrication into many product forms. Fibres made from natural sources, especially polysaccharides, have been considered the most promising due to their excellent biocompatibility, non-toxicity, and potential bioactivity at the wound surface and beyond. Many commercial wound dressing products (woven and non-woven dressings and hydrogels) are made from such natural polymers and their derivatives, the simplest being retention bandages, support and compression bandages, absorbents, gauzes, tulle dressings, and wound dressing pads produced from woven cellulose fibres (cotton and viscose) (British National Formulary, 2001; Kennedy et al., 1996 & 2001; Lloyd et al., 1998). Among the various fibrous and hydrogel products, alginate-based products are currently the most popular ones used in wound management, since they offer many advantages over traditional cotton and viscose gauzes (Horncastle, 1995; Qin and Gilding, 1996).
Recently, there has been a considerable interest in the food industry and in the preventive medicine for the development of antidiabetic and antioxidants from natural resources, such as marine flora and fauna, bacteria, fungi and higher plants. Among them, marine alga represents one of the richest sources of bioactive compounds, and algae-derived products are increasingly used in medical and biochemical research (Mayer and Lehmann, 2000). One particularly interesting feature of marine algae is their richness in sulfated polysaccharides, the uses of which span from food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Renn, 1997). These macromolecules have been proven to show a wide range of biological activities important to human health, for example, antiviral, antitumoral, antiinflammatory and anticoagulant activity (Cumashi et al., 2007; Pomin and Mourao, 2008; Ghosh et al., 2009). In recent years, several classes of sulfated polysaccharides have been demonstrated to show antioxidant activity too. The compounds tested included laminaran, alginic acid, fucoidan and other unidentified macromolecules present in the extracts (Ruperez et al., 2002; Rocha de Souza et al., 2007; Li et al., 2008).

Beneficial nutrients in seaweeds include vitamins, trace minerals, lipids, plant sterols, amino acids, and antioxidants, all of which form part of a healthy diet (Athukorala et al., 2006). They are also rich in polysaccharides such as alginates, fucans, and laminarans. Numerous studies have reported on the antioxidant capability of seaweeds or their extracts (Yan et al., 1998; Duval et al., 2000; Ruperez et al., 2002; Heo et al., 2005; Yuan and Walsh, 2006; Chandini et al., 2008). Recently, the importance of seaweeds to the pharmaceutical industry has become increasingly apparent given their established characteristics as
anticoagulants, enzyme inhibitors, and immunomodulators (Kim et al., 2006; Kim
and Joo, 2007; Athukorala et al., 2007).

Algal sulfated polysaccharides have been reported to possess diverse
biological activity of potential medicinal value, such as anticoagulant, antitumour,
anti-inflammatory, antiviral and antioxidant activity (Feldman et al., 1999). The
antioxidant activity of sulfated polysaccharide depends on several structural
parameters such as the degree of sulphation, molecular weight, other substitution
groups and position, type of sugar, and glycosidic branching. Oversulphated and
acetylated derivatives of polysaccharides exhibited higher antioxidant activity than
natural polysaccharides in vitro (Huimin et al., 2005; Qi et al., 2006). Evidence had
proven that chemical modifications of polysaccharides provided a possibility to
obtain new antioxidant agents with possible uses (Nishino and Nagumo, 1992).

To date, there are few studies on the effect of brown seaweed polysaccharide
sodium alginate on lipemia and glycemia. Here is an inverse linear relationship
between supplementary dietary fiber intake and plasma cholesterol for alginate and
an associated increase in total fecal bile levels (Seal and Mathers, 2001). Kimura
et al. (1996) examined a range of alginate formulations in rats, showing increased
cholesterol excretion and improved glucose tolerance. Parallel findings with studies
of ileostomy patients support an increase in fatty acid excretion occurring in sodium
alginate supplementation of a low-fiber diet (Sandberg et al., 1994). Wolf et al.
(2002) demonstrated a fall in peak glycemia after ingestion of a viscous alginate
drink. Williams et al. (2004) showed that incremental peak glucose was significantly
dlower and positive incremental area under the curve was significantly reduced after
consumption of an alginate-containing bar. Torsdottir et al. (1991) showed similar results in type 2 diabetic males. Sodium alginate is reported to be effective in enhancing the excretion of cholesterol (Keys et al., 1961; Tsuji et al., 1968 and 1977). Wolf et al. (2002) measured the postprandial glycemic response following ingestion of glucose based beverage containing either sodium alginate or tricalcium phosphate or a control beverage containing gum arabic and guar gum. It was found that the postprandial glycemic response was attenuated following consumption of the alginate-based glucose beverage. Williams et al. (2004) incorporated sodium alginate and dicalcium phosphate into a nutritional crispy bar and again found that following consumption of the alginate bar the postprandial glycemic response was attenuated. Both Wolf et al. (2002) and Williams et al. (2004) suggested that the effect of alginate on glucose absorption may be a consequence of reduced gastric emptying and nutrient absorption due to viscosification of gastric contents.

The brown algae harvested from the ocean are truly one of the most valuable gifts of the great deep. Sargassum polycystum is one of the marine brown algal species widely found in India, with tremendous biological applications and are known to be rich in sulfated polysaccharide content. Sulfated polysaccharides were found to possess wide pharmacological actions, especially potent free radical scavenging (Park et al., 2005) and antioxidant (Hu-Xue et al., 2001) effects. Furthermore, Raghavendran et al. (2004) have documented that the hot water extract of S. polycystum was proved to be an effective hepatoprotective agent against acetaminophen- induced liver damage. Further, Wong et al. (2000) found the S. henslowianum extract exhibited significant protection against carbon tetrachloride
induced liver injury in rats, by reducing the acute increase of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) levels.

Chemical modification of polysaccharides provided an opportunity to obtain new pharmacological agents with possible therapeutic uses. Polysaccharides have been chemically modified in various ways to change their physical or biological properties, thus allowing a broader range of applications (Liu and Sun, 2005; Xing et al., 2005). In the last decades, the biological properties of polysaccharides and their chemical derivatives, especially sulfated derivatives, have attracted much more attention (Lee et al., 2003; Han et al., 2005; Xing et al., 2005). Sulfated polysaccharides have an important bioactivities including antivirus (Urbinati et al., 2004), antioxidant (Yang et al., 2005), antitumor (Peng et al., 2005), and anticoagulant activities (Han et al., 2005). Therefore, sulfated modification may be used as a method to improve the biological activities of some polysaccharides and obtain more effective polysaccharide derivatives.

Fucoidans from brown seaweeds Eclonia cava, Sargassum hornery, and Costaria costata showed that anticancer effect on human melanoma and colon cancer cells (Ermakova et al., 2011). Human malignant melanoma cancer cell (SK-MEL-28 and SK-MEL-5) growth was inhibited by native fucoidan was isolated from Fucus evanescens (Anastyuk et al., 2012). Fucoidans from Laminaria saccharina, L. digitata, F. serratus, F. distichus and F. vesiculosus strongly blocked MDA-MB-231 breast carcinoma cell adhesion and implications in tumour metastasis (Cumashi et al., 2007). Alekseyenko et al. (2007) studied the antitumor and antimetastatic activities of fucoidan from F. evanescens in C57Bl/6 mice with
transplanted Lewis lung adenocarcinoma. Fucoidan after single and repeated
administration in a dose of 10 mg/kg produced moderate antitumor and
antimetastatic effects (Alekseyenko et al., 2007). The animals were fed with a diet
containing 1% fucoidan from Mekabu for 10 days and subcutaneously (s.c.)
inoculated with A20 leukemia cells. Thereafter, the mice were fed with the diet
containing fucoidan for 40 days. Mekabu fucoidan inhibited tumours by 65.4%
(Maruyama et al., 2006). Native and over sulfated FCSPs derived from Cladosiphon
okamuranus (Chordariales) was analyzed using ¹H NMR spectroscopy and sulfation
produced 4-mono-O-sulfo-l-fucopyranose the over sulfated FCSPs contained 2,4-di-
2-mono-and 4-mono- O-sulfo-l-fucopyranose that sulfate content and the positioning
of sulfate groups, e.g., 2,4-di- vs. 4-mono, might be important for the anti-
proliferative activity of fucoidan in a human leukemia cell line (U937) (Teruya
et al., 2007). Sulfated polysaccharides from brown seaweeds S. japonica and
Undaria pinnatifida possessed high antitumor activity and inhibit proliferation and
colony formation of breast cancer and melanoma cell lines (Vishchuk et al., 2011).

During the search for natural anticancer compounds, crude extracts and pure
compounds from marine organisms have been the object of many investigations
(Moreau et al., 2005). Over the past years, it has been reported that fucose rich
sulfated polysaccharides isolated from brown seaweeds exhibited anticancer
activity, which is one of the most important biological activities of seaweeds. Teruya
et al. (2007) reported the anti-proliferative activity of over sulfated fucoidan from
commercially cultured Cladosiphon okamuranus TOKIDA in U937 cells. Their
results indicated that the over sulfated fucoidan induced apoptosis via caspase-3 and
-7 activation-dependent pathways. In addition, fucoidan extracted from
C. okamuranus TOKIDA induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T cell leukemia cells (Haneji et al., 2005).

Alginate, a linear polymer of β-D-mannuronic acid and α-L-guluronic acid, is known to facilitate wound healing and epidermal regeneration (Wang et al., 2002). Furthermore, alginates are useful in a variety of situations. For example, sloughy wounds which also produce a degree of exudate may be dressed with alginate dressings such as Sorbsan®, Tegagen®, and Kaltostat® (or other gel forming polysaccharide dressings). The gel formed as these products absorbs exudate and forms a moist covering over the slough by preventing it from drying out (Thomas, 1997). The unique properties of sodium alginate are its biological origin, non-toxicity, hydrophilicity, biocompatibility, biodegradability and low cost makes it suitable for many biomedical applications (Sennerby et al., 1987; Li et al., 2005). Due to its good tissue compatibility, it has been widely used in the field of tissue engineering including regeneration of skin (Kong et al., 2009), cartilage (Li and Zhang, 2005), bone (Alsberg et al., 2003; Divyarani et al., 2010), liver (Yang et al., 2001; Ginzberg et al., 2003) and in the treatment of exuding wounds and in enhancing the healing process (Paul and Sharma, 2004). Considering the importance of the above findings, the present study was carried out to determine the pharmacological activities of sodium alginate extracted from brown seaweed Sargassum polycystum with the following major objectives.
Objectives

1. To extract and characterize the sodium alginate from the brown seaweed *Sargassum polycystum*.

2. To determine the antidiabetic activity of *S. polycystum* sodium alginate on alloxan induced diabetic rats and to analyze the hematological parameters, lipid profile, kidney function test, enzyme activities and reducing cholesterol and sugar.

3. To determine the *in vitro* and *in vivo* antioxidant activities of *S. polycystum* sodium alginate.

4. To screen the anticancer activity of *S. polycystum* sodium alginate by MTT assay method.

5. To analyze the antimicrobial and wound healing activities of *S. polycystum* sodium alginate on burning wound on the skin of Wistar male albino rats.
Experimental Design

Collection of brown seaweed *S. polycystum*

Extraction of seaweed polysaccharide - sodium alginate

Quantification of sodium alginate

Analysis of physicochemical parameters and molecular characterisation through FT-IR, $^{13}$C and $^1$H NMR analysis

Acute oral toxicity study of sodium alginate in rat model

Male Wistar albino rats (150 to 180g)

Intraperitoneal injection of alloxan (120mg/kg)

Sodium alginate treatment (250, 500, 750, 1000 & 1250 mg/kg) for 45 days

Analysis of blood glucose level by digital glucometer at every 3 days intervals up to 45 days

Analysis of haematological parameters, lipid profile, kidney function test, enzyme metabolism, reducing sugar and cholesterol

Antidiabetic effect

Antioxidant activity

Screening of *in vitro* antioxidant activities
1. Nitric acid scavenging activity
2. Hydroxyl radical scavenging activity

Analysis of *in vivo* antioxidant activities
1. Enzymatic antioxidant activities
   (i) Catalase
   (ii) Peroxidase
   (iii) SOD
   (iv) Glutathione peroxidase
   (v) Glutathione s-transferase
2. Nonenzymatic antioxidant activities
   (i) Reduced glutathione
   (ii) Lipid peroxidation
   (iii) L-ascorbic acid
   (iv) Nitrate level

Anticancer activity

Preparation of sodium alginate concentrates (25, 50, 100 and 250 µg/ml)

Screening of anticancer activity against four different carcinoma cell lines.
1. Mouth (HEP-2)
2. Liver (HEPG2)
3. Breast (MCF7)
4. Cervix (HELA)

Antimicrobial and wound healing effect

Screening of antibacterial activity of sodium alginate against pus forming human pathogens

Creation of burning wound on Wistar albino rats

Preparation of sodium alginate hydrogel sheet at different concentrations (100, 500 & 1000mg)

Treatment with sodium alginate hydrogel sheets and determination of wound healing percentage

Histopathological observation of new skin

Acute oral toxicity study of sodium alginate in rat model

Preparation of sodium alginate hydrogel sheet at different concentrations (100, 500 & 1000mg)

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Preparation of sodium alginate hydrogel sheet at different concentrations (100, 500 & 1000mg)

Treatment with sodium alginate hydrogel sheets and determination of wound healing percentage

Histopathological observation of new skin
2.0. GENERAL MATERIALS AND METHODS

2.1. Chapter 1: Extraction and characterization of sodium alginate from brown seaweed *S. polycystum*

The brown seaweed *S. polycystum* was collected from Kanyakumari coastal regions. The seaweed polysaccharide sodium alginate was extracted and quantified from the collected seaweed using standard procedures (modified procedure of Torres *et al.*, 2007). The purity of the extracted sodium alginate was determined by phytochemical analysis (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993). The physical characters such as colour, odour, taste, texture, particle size, moisture content and solubility of extracted sodium alginate were determined by the following methodologies of Kumar *et al.* (2011). The biochemical components such as protein, carbohydrate, lipid, fucose, total ash, acid insoluble ash, water soluble ash and sulphate contents of sodium alginate were estimated individually by following the standard procedures. The structure of the sodium alginate was predicted through UV spectral, FT-IR, $^{13}$C and $^1$H NMR analysis.

2.2. Chapter 2: Antidiabetic activity of *S. polycystum* sodium alginate on alloxan induced diabetic rats.

Uniform sizes of male Wistar albino rats were selected and the diabetic was induced by injection of alloxan at the concentration of 120 mg/kg through intraperitoneally. Seven groups of rats were maintained *viz* Group1: Normal control; Group 2: Diabetic control; Group 3 to 7: 250 to 1250mg/kg sodium alginate treated groups. After 3 days, the blood sugar level was measured and the experimental animals were treated orally with different concentrations (250, 500, 750, 1000 and
1250 mg/kg) of sodium alginate of *S. polycystum* for 45 days. During the experimental period, the blood glucose level was measured at an interval of 3 days by digital glucometer. At the final day of experiment (45th day), blood hematological parameters, blood serum lipid profiles, kidney function test, enzyme activities, reducing cholesterol and reducing sugar levels were analyzed by standard procedures.

2.3. Chapter 3: Antioxidant activity of *S. polycystum* sodium alginate on alloxan induced diabetic rats

For *in vitro* antioxidant assay, 100mg of *S. polycystum* sodium alginate was dissolved in 1ml of distilled water. From this, three concentrations such as 50, 100 and 200µl were taken for *in vitro* antioxidant studies. The nitric acid scavenging activity of sodium alginate was determined by the method proposed by Bose *et al.* (2008). Simultaneously, the hydroxyl radical scavenging activity was also determined by the methodology proposed by Yu *et al.* (2004) and Bose *et al.* (2008).

After screening of *in vitro* antioxidant activity, the *in vivo* antioxidant activities were analyzed from the kidney tissues of alloxan induced diabetic control and experimental rats treated with different concentrations of *S. polycystum* sodium alginate. On 45th day of antidiabetic study, the rats were sacrificed and the kidneys were collected from each control and experimental rats. The kidney tissues were homogenised and the cell lysates were prepared. These cell lysates were used for the analysis of various enzymatic and non enzymatic antioxidant assays such as catalase (Sinha, 1972), peroxidase (Bergmeyer, 1974), SOD (Mishra and Fridovish, 1972), glutathione peroxidase (Wendel, 1980), glutathione s-transferase (Habig *et al.*, 1974).
1973), reduced glutathione (Moron et al., 1979), lipid peroxidation (Ohkawa et al., 1979), L-ascorbic acid (Roe and Keuther, 1943) and nitrate level (Lepoivre et al. 1990) by following the appropriate methodologies.

2.4. Chapter 4: Screening of anticancer activity of *S. polycystum* sodium alginate through MTT assay

Four different carcinoma cell lines such mouth (HEP-2), liver (HEPG2), breast (MCF7) and cervix (HELA) were obtained from National Centre for Cell Science, Pune, India. Four different concentrations (25, 50, 100 and 250µg/ml) of *S. polycystum* sodium alginate were prepared and individually dissolved in 10% DMSO. The individual cell lines were treated with the above concentrations of sodium alginate. Then the viability of cells was determined by MTT assay method described by Mosmann (1983).

2.5. Chapter 5: Antimicrobial and wound healing activities of *S. polycystum* sodium alginate.

The antibacterial activities of different concentrations (10 - 100mg/ml) of *S. polycystum* sodium alginate against pus forming human pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsilla pneumoniae*, *Streptococcus pyogenes* and *Pseudomonas aerogenosa* were screened by agar well diffusion method (Cappuccino, 1986). The minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of sodium alginate were also determined by double dilution method (Lennette et al., 1974). Further, the hydrogel sheet with different concentrations (100, 500 and 1000mg) of sodium alginate were prepared as per the methodology described by Murakami et al. (2010). The
experimental rats were anesthetised with an intraperitoneal injection of ketaminexylazine (100 mg/kg) and 1 cm² burn wounds were created on the backs of the animals (Hosnuter et al., 2004). Then the rats were treated with sodium alginate hydrogel sheet and the wound closure was measured at every 3 days intervals up to 21 days. The wound healing percentage was calculated by Yates et al. (2007). At the end of the experiment (21st day), the closed wound tissues (skin) were collected from individual group of treatment and stained with hematoxylin-eosin (HE). Then the histological parameters were determined by microscopical method.

2.6. Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using one way ANOVA at 5% level of significance. Further a multiple comparison test (Tukey’s test) was conducted to compare the significant differences among the parameters using computer software Statistica 6.0 (Statsoft, UK).