Chapter-1

Introduction & Review of Literature
Microalgae

In recent years there is increased interest in functional foods, functional ingredients, nutraceuticals and other natural health products as they are recognised to promote good health, improve the state of wellbeing, and decrease risk of disease and lower health care costs by maintaining a good quality of life. (Gouveia et al 2010, Zhu et al 2016). Microalgae are acknowledged to be a very diverse source of bioactive molecules, such as phycobiliproteins, carotenoids, phenolics, flavonoids, polysaccharides, vitamins, sterols, fatty acids, etc. (Pugh 2001, Senni et al 2011, Encarnacao et al 2015, Rodrigues et al 2015, Wang et al 2016). In the last three decades their potential as a unique source of biochemical molecules of value in food, cosmetics, feed and pharmaceutical sectors has been realized (Borowitzaka 1995, Sousa 2008, da Silva Vaz et al 2016).

Of these few algae like Chlorella vulgaris and Spirulina are exploited commercially used in human nutrition, mainly as food supplements in health food market (Moore 2001).

Microalgal metabolites

Microalgae are miniature biochemical factories, providing a great diversity of primary and secondary metabolites, (Table 1.1). Some of them are of high commercial value (Spolaore 2006, Plaza 2008, Plaza 2009). These include pigments (Carotenoids), fatty acids, proteins, polysaccharides, vitamins and minerals, sterols etc.
### Table 1.1 Potential Functional Ingredients Found in Different Microalgae and their Bioactivity

<table>
<thead>
<tr>
<th>Functional ingredients</th>
<th>Health benefits</th>
<th>Microalgae</th>
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<tr>
<td><strong>Carotenoids</strong></td>
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<tr>
<td>β-carotene</td>
<td>antioxidant activity</td>
<td><em>Dunaliella salina,</em> <em>Haematococcus pluvialis</em></td>
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<tr>
<td>astaxanthin</td>
<td>antioxidant, immunomodulation, and cancer prevention</td>
<td><em>H. pluvialis, Chlorella vulgaris</em></td>
</tr>
<tr>
<td>canthaxanthin</td>
<td>antioxidant, immunomodulation, and cancer prevention</td>
<td><em>C. vulgaris, H. pluvialis</em></td>
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<tr>
<td>lutein</td>
<td>antioxidant activity</td>
<td><em>Chlorella Pyrenoidosa,</em> <em>H. pluvialis</em> ellipsoidea</td>
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<tr>
<td>violaxanthin</td>
<td>antioxidant activity</td>
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<td><strong>Fatty acids</strong></td>
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<tr>
<td>EPA fatty acid</td>
<td>EPA fatty acids reduce risk of certain heart diseases</td>
<td><em>Phaeodactylum</em></td>
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<td></td>
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<td><em>Tricornutum, Monodus</em></td>
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<td></td>
<td></td>
<td><em>Subterraneus, Porphyridium cruentum</em></td>
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<tr>
<td>oleic acid</td>
<td>antioxidant activity</td>
<td><em>H. pluvialis, C. vulgaris,</em> <em>D. salina, Spirulina platensis</em></td>
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<tr>
<td>linolenic acid</td>
<td>antimicrobial activity</td>
<td><em>D. salina, S. platensis</em></td>
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<tr>
<td>palmitic acid</td>
<td>antimicrobial activity</td>
<td><em>D. salina</em></td>
</tr>
<tr>
<td>palmitoleic acid</td>
<td>reduce risk of certain heart diseases</td>
<td><em>S. platensis</em></td>
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<tr>
<th>Proteins</th>
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<th>S. platensis, Porphyridium spp.</th>
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<tbody>
<tr>
<td>phycobiliproteins</td>
<td>immunomodulation activity, anticancer activity, and hepatoprotective, anti-inflammatory, and antioxidant properties</td>
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<table>
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<tr>
<th>Polysaccharides</th>
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<th>C. pyrenoidosa, Porphyridium spp.</th>
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<tbody>
<tr>
<td>sulfated polysaccharide</td>
<td>antiviral, antitumor, antihyperlipidemia, and anticoagulant</td>
<td></td>
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<tr>
<td>insoluble fiber</td>
<td>reduce total and LDL cholesterol</td>
<td>C. vulgaris</td>
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<tr>
<th>Vitamins</th>
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<th>Porphyridium spp., S. platensis</th>
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<tr>
<td>tocopherols (vitamin E)</td>
<td>antioxidant activity</td>
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<tr>
<th>Phenolic compounds</th>
<th>antioxidant activity</th>
<th>S. platensis</th>
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<tbody>
<tr>
<td>benzoic acid derivatives, hydroxybenzaldehydes, and cinnamic acid derivatives</td>
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<thead>
<tr>
<th>Volatile compounds</th>
<th>antimicrobial activity</th>
<th>S. platensis, D. salina, Phormidium</th>
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<tr>
<td>neophytadiene, phytol, etc.</td>
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(Modified Plaza et al 2008)
Pigments

Microalgae being photosynthetic microorganisms have a set of light harvesting molecules which include carotenoids. These carotenoids are found to have great health and therapeutic potential. (Dufosse et al 2005, Mimouni et al 2012)

Carotenoids

Different colours of fruits, vegetables and other plants are due to presence of pigments. The important natural pigments of high nutrition value present in fruits and vegetables are carotenoids (Ben-Amotz and Fishler 1998).

Microalgae are photoautotrophic organisms and are exposed to high oxygen and radical stresses consequently they have developed several efficient protective systems against reactive oxygen species and free radicals (Pulz and Gross, 2004). Microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity which is much more diverse than higher plants. The value of microalgae as a source of natural antioxidants is further enhanced by the relative ease with which target compounds can be purified (Safafar et al 2015). Some of the important carotenoids which include β-carotene, astaxanthin, canthaxanthin, lutein are listed in Table 1.2

Table 1.2 Microalgae producing carotenoids

<table>
<thead>
<tr>
<th>Microalga source</th>
<th>Active compound</th>
</tr>
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<tbody>
<tr>
<td><em>Dunaliella salina</em></td>
<td>β-carotene</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>Astaxanthin, canthaxanthin, lutein</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>Canthaxanthin, astaxanthin</td>
</tr>
<tr>
<td><em>Coelastrella striolata var. multistriata</em></td>
<td>Canthaxanthin, astaxanthin, β-carotene</td>
</tr>
<tr>
<td><em>Scenedesmus almeriensis</em></td>
<td>Lutein, β-carotene</td>
</tr>
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</table>
Currently, microalgal phytochemicals are considered to be a promising source of natural antioxidants to replace synthetic antioxidants for food applications (Rao et al. 2006; Li et al. 2007).

![Figure 1.1 Structure of the important carotenoids of microalgae](image)

**Figure. 1.1 Structure of the important carotenoids of microalgae**

Structures of important carotenoids of microalgae as shown in Figure 1.1. Antioxidants are involved in enhancing the shelf life of foodstuffs, mainly by retarding lipid oxidation in food and chemical industries (Maqsood et al. 2013). Carotenoids rich diet has been recognised to reduce the risk of cardiovascular disease as it lowers the prevalence of metabolic syndrome, adiposity and serum triglyceride concentrations in middle-aged and elderly men (Sluijs et al. 2009). It is also reported that it diminishes the prevalence of certain types of cancer, such as (breast and lung cancer), atherosclerosis, cataracts, age-related muscular degeneration; and also carotenoids reported to have antimicrobial activity (Gouveia et al 2010).

The main carotenoids produced industrially by microalgae are β-carotene from* Dunaliella salina* and astaxanthin from* Haematococcus Pluvilalis*. Astaxanthin gets accumulated up to 3.8% on the dry weight basis in* H. Pluvilalis* (Ambati et al 2014).
In general, astaxanthin is used as a dietary supplement for its potential health benefits (Stewart et al. 2008).

Astaxanthin involved in protecting from skin cancer (Rao et al 2013) from premature ageing and reducing inflammation and UVA damage. Isolated astaxanthin (ASX) and astaxanthin esters (ASXEs) from green microalga *Haematococcus pluvialis* were found to have hepatoprotective activity (Rao et al 2015).

β-Carotene is both recognised as safe and as having positive health effects because of its provitamin A activity. It serves as an essential nutrient and is in high demand and commercially used in health food market, cosmetics etc (Raja et al. 2007).


**Proteins**

Microalgae as source of protein was of great interest because of the high protein content of various microalgal species including *Spirulina*. (Becker 2007).

**Phycobiliproteins**

Of the diverse compounds produced by microalgae, the algal pigments such as phycobiliproteins have gained commercial importance owing to their unique and multiple applications as food, cosmetic colorants, antioxidants, nutraceuticals and pharmaceuticals

Besides chlorophyll and carotenoids, cyanobacteria (blue-green algae), rhodophyta (red algae) and cryptomonad algae contain lipophilic pigments (phycobiliproteins). These pigments are deep coloured water-soluble, fluorescent molecules and also they
are major components of a complex assemblage of photosynthetic light-harvesting antenna pigments i.e phycobilisomes (Glazer 1994).

**Structure and classification of phycobiliproteins**

The phycobiliproteins consists of protein moiety and linear tetrapyrrole prosthetic group (phycobilins) attached to the polypeptide through thioether bonds. Based on spectroscopic properties these proteins are broadly classified into 3 groups- allophycocyanin (650-655nm), C-phycocyanin (610-620nm) and R-phycoerythrin (540-570nm), each of these phycobiliproteins are comprised of 2 sub units α and β to which linear tetrapyrrole are covalently attached by a cysteine thioether bond. The central portion of all the 3 groups is an open tetrapyrrole containing skeletal system similar to chlorophyll and haemoglobin. Four types of tetrapyrroles are known phycocyanobilin, (as shown in Figure 1.2), phycoerythobilin, phycourobilin and phycoerythrocyanin. These chromophoretic proteins along with some of the colourless linker peptides form an organized structure called phycobilisome (De Marsac and Bazire 1977).

![Figure 1.2 Structure of Phycocyanobilin](image)

Purified phycocobiliproteins may exist as monomers (α, β) or dimmers or trimers or hexamers or equilibrium mixtures of two or more of these aggregates depending on
pH, ionic strength, solvent composition, protein concentration and temperature. These proteins are stable over a pH range of 5.0 to 7.5 (Sarada et al 1999)

**Biological activities of phycocyanin**

Due to increasing awareness of the environmental hazard of synthetic colors, biological sources of natural colors have been in great demand. C-Phycocyanin is used as bioactive molecule in food, cosmetics and diagnostics. Some of the applications of phycocyanin are listed in (Table 1.3).

**Table 1.3 Biological activities of phycocyanin in vitro and in vivo models**

<table>
<thead>
<tr>
<th>Biological Activities</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Ameliorative</td>
<td>Hussein et al 2015</td>
</tr>
<tr>
<td>Antiallergic</td>
<td>Liu et al 2015</td>
</tr>
<tr>
<td>Selenite induced cataractogenic rat model</td>
<td>Kumari et al 2015</td>
</tr>
<tr>
<td>Monosodium glutamate induced Oxidative stress</td>
<td>Bertrolin et al 2011</td>
</tr>
<tr>
<td>Photodynamic</td>
<td>Cai et al 2014</td>
</tr>
<tr>
<td>Immunomodulatory</td>
<td>Chen et al 2014</td>
</tr>
<tr>
<td>Oxalate mediated oxidative stress</td>
<td>Farooq et al 2014</td>
</tr>
<tr>
<td>Oxalate mediated renal cell injury</td>
<td>Farooq et al 2004</td>
</tr>
<tr>
<td>Cisplatin induced nephrotoxicity oxidative stress</td>
<td>Fernandez-Rojas et al 2014</td>
</tr>
<tr>
<td>Induced apoptosis</td>
<td>Gantar et al 2012</td>
</tr>
<tr>
<td>Antitumor</td>
<td>Gardeva et al 2014, Li et al 2015</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>Hsiao et al 2005, Chiu et al 2006</td>
</tr>
<tr>
<td>Salicylate induced tinnitus</td>
<td>Hwang et al 2013</td>
</tr>
<tr>
<td>Sodium selenite mediated cataractogenesis</td>
<td>Kumari and Anbarasu 2014</td>
</tr>
<tr>
<td>Beta cell apoptosis</td>
<td>Li et al 2014</td>
</tr>
<tr>
<td>Higher efficacy of in vitro antioxidant activity when exposed to blue light</td>
<td>Madhyastha et al 2009</td>
</tr>
<tr>
<td>Thermal stabilityyn</td>
<td>Martelli et al 2014</td>
</tr>
</tbody>
</table>
Toxicity assessment Naidu et al 1999
Cell therapy against oxidative stress Park et al 2014
Human erythrocytes Pleonsil et al 2013
Antibacterial and toxicity assessment Sabarinathan and Ganesan 2008
Colon carcinogenesis Saini and Sanyal 2012
Cyclooxygenese-2 inhibitor Reddy et al 2000
Phagocytic Satyantini et al 2014
Anti-proliferative Tantirapan and Suwanwong 2014, Thangam et al 2013
Inhibitory effect on growth of HeLa cells in vitro Yang et al 2014
Anticancer Zhang et al 2011
Antioxidative, antiproliferative and neuroprotective Romay et al 2003,
Peroxyle radical scavenging activity Bhat and Madhysthata 2000, Deng et al 2010
Antinflammatory Romay et al 2003
Hepatoprotective Ou et al 2010, Gad et al 2011

Purified C-PC reported to have both nutraceutical and pharmaceutical potential. Recently its applications are used in biosorption of azo dyes from the industrial effluents (Kuddus et 2013, Deniz et al 2016, Zinicovscaia et al 2016).

Fatty acids

Algal fatty acids are either saturated, mono-unsaturated or polyunsaturated acids (PUFAs), which are essential to human and animal nutrition and are used in food sources. Some of the important fatty acids are arachidonic acid (AA) and eicosapentaenoic acid (EPA) and DHA. Commercially produced microalgal fatty acids of particular interest are EPA (rheumatoid arthritis, and treatment of heart disease), Docosahexaenoic acid (DHA) (anti-inflammatory) and palmitoleic acid

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(reduce the risk of certain heart diseases), oleic acid (antioxidant capacity), linolenic acid and palmitic acid (anti-microbial activity) (Otles et al 2001, Stengel et al 2011)

**Vitamins and minerals**

Studies reported that microalgal biomass as a rich source of all essential vitamins (Brown 1999, Becker 2004).

**Vitamin B\(_{12}\)**

Few microalgae such as *Spirulina*, *Chlorella* and *Dunaliella* and *Nori*, a seaweed were also reported to contain significant amount of vitamin B\(_{12}\) (Takenaka et al. 2001; Kumudha et al. 2010, Kumdha et al 2015, Kumudha and Sarada 2016).

**Tocopherols**

Microalgae have been reported to contain rich source of tocopherols (Mendiola et al 2008, Kalogeropoulos et al 2010, Durmaz et al 2007, Durmaz 2007). Tocopherols always have been considered as an essential constituent of dietary supplements/functional foods. They are important bioactive molecules due to their and ability to protect DNA, protein amino acids and lipids (PUFAs) from oxidative damage by neutralising free radicals by initiating chain reactions (Gouveia et al 2010).

**Sterols**

Many polyhydroxysterols from marine organisms have anti-cancer, cytotoxic and other biological activities. (Permeh et al 2012, Kalogeropoulos et al 2010). Since phytosterols reduce the level of cholesterol in the blood, they are valuable as constituents of functional foods, Gouveia et al 2010)
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Hypertension

Wold health organisation (WHO) estimated that the number of adults with hypertension will reach 1.56 billion people by 2025 (Zhao et al 2015). People suffering from hypertension is associated with stroke, arteriosclerosis and kidney related disorders (Je et al 2005).

In India about 33% urban and 25% rural Indians are hypertensive. Of these, 25% rural and 42% urban Indians are aware of their hypertensive status. Only 25% rural and 38% of urban Indians are being treated for hypertension. (Anchala et al 2014)

Renin-angiotensin system (RAS)

Blood pressure regulation has been connected with the renin-angiotensin system (RAS). The renin angiotensin system plays a vasodilator mechanism and try to absorb renal sodium to maintain blood pressure (Figure. 1.3)

![Renin-angiotensin system (RAS)](http://cvpharmacology.com/vasodilator/ACE)

**Fig 1.3 Renin-angiotensin system (RAS)**
Further ACE (Angiotensin-I converting enzyme) converts the inactive decapeptide angiotensin-I to potent vasoconstricting octapeptide angiotensin II (Figure 1.4). This potent vasoconstrictor is mainly involved in the release of a sodium-retaining steroid, aldosterone, from the adrenal cortex, which has a tendency to increase blood pressure. ACE (dipeptidyl carboxpeptidase, EC 3.4.15.1) is a zinc metallopeptidase. ACE is generally distributed in mammalian tissues, mainly as a membrane-bound ectoenzyme. ACE is present in vascular endothelial, absorptive epithelial, neuroepithelial, and male germinal cells.

Inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. A large number of commercial synthetic inhibitors available in the market are used in the treatment of hypertension and these synthetic inhibitors found to show highly potent and specific for ACE.
Inhibitors/Drugs for ACE

Presently there are different synthetic/inhibitors/drugs for ACE in clinical use (Zaman et al 2002). The structures of these synthetic drugs are shown in Figure 1.5

![Chemical structures of commercial ACE inhibitors/Drugs](image)

**Figure 1.5 Chemical structures of commercial ACE inhibitors/Drugs**

**Side effects**

Synthetic ACE inhibitors although remarkably effective they are reported to have side effects which include dry, persistent cough, sore or swollen throat, abdominal pain, constipation, kidney and liver problem. (Koh, 2009).

As there is much concern about side effects of synthetic drugs there is increasing interest for developing safer and natural ACE inhibitors.
Angiotensin I converting enzyme (ACE) inhibitors from microalgae.

Antihypertensive peptides are inactive in the original protein forms, and can be converted to active forms through hot water treatment, organic solvent extraction, proteases hydrolysis, and microbial fermentation process.

Some of the ACE inhibitory molecules derived from microalgae are shown in Figure 1.6. The methods of heating and organic solvent extraction to produce ACE-inhibitory peptide would greatly damage proteins, and cause environmental pollution. The best method is to obtain bioactive peptides by proteolytic hydrolysis where peptides remain intact retaining their structure and function and are stable. (He et al 2013).

Microalgae including *Spirulina platensis*, *Chlorella vulgaris* *Nannochloropsis oculata*, *Isochrysis galbana* were reported to have ACE-inhibitory peptides (Lu et al
In the last decade, a large number of ACE-inhibitory peptides have been discovered from marine protein hydrolysates, such as Alaska pollack skin gelatin, bigeye tuna dark muscle, shrimp oyster, microalgae (*Chlorella vulgaris* and *Spirulina*), shark meat, shrimp yellowfin (Hai-Lun et al 2006, Kim and Wijesekara 2010).

Phlorotanins derived from brown alga were reported to have ACE inhibitory activity (Wijesinghe et al et al 2011). Previous studies on ACE inhibitory activity from microalgae was limited to phlorotanins and some bioactive peptides derived from crude protein. In order to understand the mechanism of action further research is required on purified/synthesized molecules. Many studies on phenolic and flavonoid compounds extracted from different plant and animals sources were show to have ACE inhibitory activity (Clemente et al 1999, Park and Jhon 2010, Ojeda et al 2010, Liu et al 2010, Massaretto et al 2011, Shukor et al 2013, Zouari et al 2011, Ahmed et al 2010, Cheplick et al 2010, Sreerama et al 2012, Balasuriya and Rupasinghe 2011, Makinen et al 2012). Studies also show that microalgae also are rich source of phenolic, flavonoid and bioactive peptides (El-Baky et al 2009, Wu et al 2005, Wang et al 2007, Lu et al 2010, Suetsuna and Chen 2001, Kepekci et al 2012).

In order to explore the potential of these bioactive molecules from microalgae for using them in place of synthetic molecules the study and investigate the extraction and purification of phenolic, flavonoids and bioactive peptides.

**Diabetes mellitus**

Diabetes mellitus is a chronic disease and is the major health problem in modern society. In fact the global prevalence of diabetes in all age groups was estimated to be
171 million (2.8%) in 2000 and projected to reach 366 million (4.4%) in 2030. (Rathmann and Giani et al 2004).

In India more than 62 million diabetic individuals currently diagnosed with the disease. It is reported that in the year 2000 India topped the world with the highest number of people with diabetes mellitus followed by China, United States (Kaveeshwar and Cornwall 2014). Type 2 diabetes mellitus is associated with significant health disorders. Maintaining blood glucose levels of normal range will significantly lowers the risk of complications and is an important therapeutic approach. Presently number of oral antihyperglycemic drugs are available in the market to treat diabetes by lowering blood glucose concentrations. Structures of the some of the oral antihyperglycemic synthetic drugs presently in use are shown in Figure 1.7

![Figure 1.7 Structure of the commercial antihyperglycemic drugs.](image)

**Metformin**  **Glimepride**  **Acarbose**  **Voglibose**
Side effects of synthetic drugs for diabetes

Presently synthetic drugs such as acarbose are used in the treatment for diabetes, although acarbose is effective in treating diabetes it is associated with adverse side effects on gastrointestinal system (Chiasson et al 2002). α-glucosidase inhibitors can hinder the release of D-glucose from carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppression of postprandial hyperglycemia.

Alpha glucosidase inhibitors

α-Glucosidase is distributed widely in microorganisms, plants, and animal tissues. It is a membrane bound enzyme present in the epithelium cells of the small intestine. It catalyz the hydrolytic cleavage of oligosaccharides into absorbable monosaccharaides such as glucose in the small intestine. (Figure 1.8).

![Conversion of oligosaccharide to glucose](image)

**Figure 1.8. Conversion of oligosaccharide to glucose (Kumar et al 2011)**

Inhibition of α-glucosidase is one of the therapeutic approaches to decrease the postprandial hyperglycemia in the intestine and is proved to be one of best strategies to treat postprandial hyperglycemia for avoiding the diabetic complications.
Phlorotanins such as phloroglucinol, dioxinodehydroeckol, eckol, dieckol and phloroeckol derived from edible brown algae, *Ecklonia stolonifera* and *Eisenia bicyclis* were reported to have α-glucosidase inhibitory activity (Moon et al 2011, Lee and Jeon 2013, Lee et al 2009). Purified bromophenols such as 2,4,6 tribromophenol and 2,4-dibromophenol extracted from red alga *Gratteloupia elliptica* and bromophenol, bis (2,3-dibromo-4,5-dihydroxybenzyl) ether from the red alga *Polyopes lancifolia* were show to have α-glucosidase inhibitory activity (Kim et al 2008, Kim et al 2010, Liu et al 2011).

![2,4-Dibromophenol](image1.png) ![2,4,6-Tribromophenol](image2.png)

**Figure 1.9. α-glucosidase inhibitory molecules from microalgae**

Phenolic rich extracts derived from macroalgae *Palmaria, Ascophyllum* and *Alaria* were also found to have α-glucosidase inhibitory activity (Nwosu et al 2011).
Fucoidan a water soluble polysaccharide extracted from two algal species (*Ascophyllum nodosum* and *Fucus vesiculosus*) found to have $\alpha$-glucosidase inhibitory activity (Kim et al 2014).

Literature survey indicated that $\alpha$-glucosidase inhibitors from microalgae was only limited to phlorotanins and bromophenols. However in addition to these phlorotanins microalgae contain phenolic, flavonoids and bioactive peptides which are unexplored (El-Baky et al 2009, Wu et al 2005, Wang et al 2007, Lu et al 2010, Suetsuna and Chen 2001). Previous studies on phenolic and flavonoid compounds extracted from different plant sources were shown to have $\alpha$-glucosidase inhibitory activity. Bhattacherjee et al (2014) reported antioxidant, $\alpha$-glucosidase and antimicrobial activity of methanolic extract from tropical weed (*Alternanthera philoxeroides*) which contain phenolic compounds such as kaempferol, ferulic acid, salicylic acid, syringic acid and chlorogenic acid. Ethyl acetate extract of Carols muscadine which was found have ellagic acid and quercetin exhibited $\alpha$-glucosidase inhibitory activity (You et al 2012). Bioactive peptides extracted from different plant and animal sources reported to have inhibitory activity against $\alpha$-glucosidase enzyme (Yu et al 2012, Ren et al 2016, Zhang et al 2016, Connolly et al 2014, Yu et al 2011).

Few clinical studies reported on antidiabetic activity of *Spirulina* biomass and phycocyanin in rat models (Layam and Reddy 2006, Muthuraman et al 2009, Ou et al 2013, Pankaj and Verma 2013). In order to understand the mechanism of action and active principals involved the present investigation carried out the purification and characterization of bioactive molecules like phenolic, flavonoids, peptides and carotenoids from microalgae.
Lipoxygenases (LOXs)

Lipoxygenases (LOXs) (linoleate: oxygen oxidoreductase EC 1.13.11) are a family of none-heme, non-sulphur containing deoxygenases that are involved in the generation of hydroxyeicosatetraenoic acids and leukotrienes (LTs) (Sudha and Srinivasan 2014).

**LOXs as pharmacological target**

As 5-LOX metabolizes arachidonic acid to leukotrienes, it is regarded as a potent target for drugs of many diseases. In mammals, the product of LOXs-catalysed reactions are responsible for variety of human disorders such as atherosclerosis, allergy, inflammation, asthma and hypersensitivity (Steinburg 1999, Prigge et al., 1997, Funk and Colin 2006). The inhibition of 5-lipoxygenase pathway is considered to be important in the treatment of a variety of inflammatory diseases (Figure 1.10). Besides 5-lipoxygenase inhibitors are also show potential against cyclooxygenase enzymes and can be used as therapeutic value. (Schneider and Bucar 2005).

![Figure 1.10. Lipoxygenase catalysed arachidonic acid pathway](http://www.asthma.partners.org/NewFiles/IsraelAspirinSensitiveAsthma.html)
Anti-leukotriene drugs

Currently pharmacological agents that specifically inhibit the LOX-mediated signalling pathways are commercially available to treat inflammatory diseases such as asthma, arthritis and psoriasis (Figure 1.11). Major targets are aimed at inhibition of LO activity (5-LO and associated or 12-LO and leukotriene receptor antagonism.

Figure 1.11 Anti leukotriene drugs
Side effects of anti leukotriene drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicines for treating inflammation and associated diseases. Even though they are very useful for the inflammatory disorders, their prolonged use may cause severe adverse side-effects. Gastrointestinal bleeding and peptic ulcers are the most common of these side-effects. (Charlier and Michaux 2003). The present study focus on developing natural anti-inflammatory agents like phenolic flavonoids, bioactive peptides and other bioactive molecules from microalgae to combat the side effects of synthetic drugs.

Lipoxygenases inhibitors from microalgae

Phlorotanins such as eckol, dieckol, 6, 6–bieckol are extracted from brown alga were reported to have significant 5-lipoxygenase inhibitory activity (Shibata et al 2003, Vo et al 2012, Kurihara et al 2015).

![Lipoxygenase inhibitory molecules from microalgae](image)

**Figure 1.12.** Lipoxygenase inhibitory molecules from microalgae
Introduction and review of literature

Structures of some of the lipoxygenase inhibitors from microalga are shown in Figure 1.12. Literature survey indicated that studies on lipoxygenase inhibitory molecules was derived from microalgae are limited to phlorotanins. However other than phlorotanins microalgae contain phenolic, flavonoids and bioactive peptides which are unexplored (El-Baky et al 2009, Wu et al 2005, Wang et al 2007, Lu et al 2010, Suetsuna and Chen 2001).

Studies reported that these bioactive molecules extracted and purified from different plant and animal sources were found to have lipoxygenase inhibitory activity. Studies showed that synthesised coumarins (on position 3 a substituted phenyl ring) found to have 5-lipoxygenase inhibitory activity (Roussaki et al 2010), Phenolic constituents such as luteolin, caffeic acid, protocatechuic acid from the flower buds Lonicera japonica found to have 5-lipoxygenase inhibitory activity (Lee et al 2010). Flavonoids such as catechin, gallocatechin, trihydroxyflavone derived from S. subberctus were reported to have 5-lipoxygenase inhibitory activity (Jiang et al 2015). Bioactive peptides extracted and purified from tryptic β-casein digest was shown to have 5-lipoxygenase inhibitory activity (Rival et al 2001).

In order to explore these bioactive molecules the present investigation studied with extraction and purification of phenolic, flavonoids and bioactive peptides from selected microalgae for screening for lipoxygenase inhibitory activity.

Antimicrobial activity

Resistant microbes are able to cause infections that are more difficult to treat and more expensive; some strains have become resistant to all available antimicrobial agents (Byarugaba, 2004). Resistant infections affect treatment costs, disease spread and duration of illness (Spratt 1994, Okeke et al., 2005).
Natural occurring antimicrobial compounds

Bioactive molecule extracted from natural sources may overcome the undesirable side effects caused by synthetic molecules (Saleem 2010, Maobe et al 2013). Structures of some of the important antimicrobial compounds produced by microalgae are shown in Figure 1.13.

![Phytol](image1.png)
![Linalool](image2.png)
![Eugenol](image3.png)
![Carvacr](image4.png)

**Figure 1.13. Antimicrobial compounds produced by microalgae**


As stated microalgae are also a rich source of these bioactive molecules and therefore the present investigation studied extraction and purification of phenolic, flavonoids
and bioactive peptides from selected microalgae for screening for antimicrobial activity.

**Aim and scope of the present investigation**

*You are what you eat. Let thy food be they medicine*” Hippocrates (460-377 BC)

It is life style that has foremost role to play in today’s disorders and major life style related disorders are hypertension and diabetes.

Algal bioactive molecules may prove to be a natural source for treating these life style related disorders. Microalgae have been valued for high nutritional content for decades. Nutritional issues highlight the relationship between diet and chronic diseases. Current attention focus on ACE, α-glucosidase, lipoxygenase, antioxidant and antimicrobial inhibitory molecules from microalgal sources that are promising natural bioactive molecules which are alternatives to synthetic drugs. From this perspective, the development of new food products is a must. Besides nutritional and energy issues, functional ingredients and foods must be targeted for the prevention and treatment of hypertension, diabetes and inflammatory diseases. To date there is limited information available on the relationship between structure and activity of bioactive molecules derived from microalgal source. Literature survey indicated that studies on ACE, α-glucosidase, lipoxygenase inhibitory molecules from microalgae are limited to phlorotanins and peptides derived from crude protein. However other than phlorotanins microalgae contain phenolic, flavonoids and bioactive peptides which are unexplored (El-Baky et al 2009, Wu et al 2005, Wang et al 2007, Lu et al 2010, Suetsuna and Chen 2001). In order to understand the mechanism of action it needs a further investigation on extracted purified/synthesized molecules. Presently industrial products from microalgae are developed from their biomass and a lot of spent cell media used for mass cultivation that is unexploited, which may contain an
excellent source of potent bioactive molecules for further use (Liu et al 2016). It is evident that, the extracellular metabolites derived from spent media of microalgae have been neglected. Some of these extracellular metabolites can be extracted, purified and their potential can be screened for ACE, α- glucosidase, and lipoxygenase inhibitory activities. Inhibition of these enzymes can significantly control over blood pressure, postprandial hyperglycemia and inflammatory associated disorders. Thus these extracellular metabolites can be applied in functional foods/food formulations. Extraction and purification of bioactive molecules from microalgae is facilitates to understand the mechanism of action of molecules in the lowering of hypertension, lowering of sugar levels, antioxidant and anti-inflammatory. Literature survey indicated that microalgae can provide an enormous number of important bioactive molecules (functional ingredients), and their incorporation in traditional foods may turns them into functional foods with healthy benefits without changing food habits.

Based on the above information the objectives were designed. The main objectives of the present investigation are:

1. Purification and molecular characterization of bioactive active molecules from selected microalgae.
2. Evaluation of biological activities of purified biomolecules for Angiotensin-1 converting enzyme and α-Glucosidase, Lipoxygenase inhibitory activities, antioxidant and antimicrobial activities.
3. Evaluation of selected bioactive molecule(s) for α-Glucosidase inhibitory properties in suitable in vivo experimental models.