Diseases of the thyroid gland are among the most abundant disorders worldwide secondary to diabetes (Mohanty et al., 2008). Thyroid function needs to be assessed by measuring hormones produced by the gland, i.e. $T_3$ and $T_4$ and also the pituitary hormone, i.e. TSH. Thyroid disease should be diagnosed and treated well in order to prevent other health problems. Successful treatment of thyroid disorder requires normalizing thyroid hormone levels in peripheral tissues with the use of replacement therapy in case of hypothyroidism. Hyperthyroidism can be treated by medication to block the effects of excessive production of thyroid hormone and treatment with radioactive iodine to destroy the thyroid gland or surgical removal of the thyroid gland (Lee, 2010).

The commonly used drug for the treatment of hypothyroidism is levothyroxine sodium (a form of thyroxine). Hyperthyroidism is treated using antithyroid drugs such as neomercazole, carbimazole, methimazole and propylthiouracil (Reid and Wheeler, 2005). During the course of the treatment hypothyroid patient may become hyperthyroid and vice-versa which may lead to other health complications. Although very less reports are available on the adverse effects of thyroid medication, some of the side effects of the antithyroid medications include a potentially fatal reduction in the level of white blood cells, agranulocytosis, granulocytopenia, aplastic anemia, fulminant liver failure, rashes and peripheral neuritis (Bal and Chawla, 2010). Due to this some patients may stop their medication and opt for herbal medicine.

Traditional system of medicine continues to be widely practiced on many accounts. Plant materials have been used as medicine for a wide variety of human ailments due to increase in cost of treatment, side effects of several allopathic drugs and development of resistance to currently used drugs for
infectious diseases. Therefore, usage of herbal form of medicine will be more helpful, economical and with less side effects (Nayak et al., 2010).

Hence in the present study a survey was carried out to find the prevalence of the hypo and hyperthyroidism in selected hospitals and to study the treatment regimen and complications during medication. The effects of selected seaweed and plant sample were analyzed in experimental animals by inducing hypo and hyperthyroidism. Phytochemical constituents of the selected samples were also determined.

The experimental procedure for the present study entitled “Influence of drug therapy in thyroid disorder patients and the effect of Sargassum wightii Greville and Maydis stigma on experimentally induced hypo and hyperthyroidism in Swiss albino rats” was studied under three phases.

Phase I

3.1 Study the Prevalence of Hypo and Hyperthyroidism in Selected Hospitals

This phase included the survey and the study of the prevalence of hypo and hyperthyroidism in selected hospitals of Coimbatore city. Biochemical and Clinical changes were studied in selected hypo and hyperthyroid patients who were undergoing treatment.

3.1.1 Survey of Patients with Clinical History and Symptoms
3.1.2 Screening for Thyroid Disorders
3.1.3 Biochemical and Clinical Parameters Analyzed in the Selected Patients

Phase II

3.2 Phytochemical Analysis of the Selected Seaweed and the Plant Sample

This phase consisted of selection of seaweed (brown algae) and medicinal plant (Zea mays L.) for the study and its phytochemical analysis
3.2.1 Selection of Seaweed (brown algae) and Medicinal Plant (Zea mays L.) for the Study

3.2.2 Collection and Preparation of the Selected Samples

3.2.3 Estimation of Iodine Content in the Study Samples

3.2.4 Qualitative Analysis for the Presence of Phytochemicals

3.2.5 Determination of Free Radical Scavenging Activity (DPPH Scavenging Activity) in the Selected Samples

3.2.6 Evaluation of the Total Antioxidant Activity in the Selected Samples

3.2.7 Characterization of Bioactive Components Present in the Study Samples using HPLC, HPTLC, GC-MS, UV-Visible and FT-IR Analysis

Phase III

3.3 Animal studies

To find out the effect of methanolic extract of Sargassum wightii Greville (brown algae) and Maydis stigma (Zea mays hair) on experimental animals, in vivo studies were carried out.

3.3.1 Selection and Maintenance of the Animals

3.3.2 Grouping of the Animals

3.3.3 Weight of the Experimental Animals

3.3.4 Biochemical Parameters Analyzed in Experimental Animals

3.3.5 Induction of Experimental Hypo and Hyperthyroidism

3.3.6 Treatment of the Animals with Standard Drugs

3.3.7 Treatment of the Animals with Sample Extracts

3.3.8 Histopathological studies

3.4 Statistical Analysis
Experimental Procedure

Phase I

3.1 Study the Prevalence of Hypo and Hyperthyroidism in Selected Hospitals

3.1.1 Survey of Patients with Clinical History and Symptoms

It is important to know the patient’s medical history and symptoms before getting into the conclusion of any particular disease or disorder. One disorder can give rise to another disorder in future if it is not diagnosed and treated properly. The clinical history and symptoms of the patients were collected by personal interaction and through questionnaire method (Appendix I and II).

3.1.2 Screening for Thyroid Disorders

Patients complaining with sign and symptoms of thyroid disorders were screened for the thyroid hormone level. The study was carried out in three selected hospitals of Coimbatore city for a period of one year. For the screening of the thyroid hormone level, 3ml of venous blood was collected from the selected patients after 12 hours of overnight fasting. Blood samples were centrifuged and serum was separated. With the serum assays were performed.

Table 1

Parameters analyzed in patients screened with thyroid disorders

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Method of analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triiodothyronine (T&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>ELISA</td>
<td>Wisdom (1976)</td>
<td>III</td>
</tr>
<tr>
<td>2</td>
<td>Thyroxine (T&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>ELISA</td>
<td>Wisdom (1976)</td>
<td>IV</td>
</tr>
<tr>
<td>3</td>
<td>Thyroid Stimulating Hormone (TSH)</td>
<td>ELISA</td>
<td>Soos and Siddle (1982)</td>
<td>V</td>
</tr>
</tbody>
</table>

The parameters analyzed and their method of analysis in patients screened with thyroid disorders are indicated in table 1.
Triiodothyronine ($T_3$) and thyroxine ($T_4$) are two active hormones found in the bloodstream. Total serum $T_3$ is one parameter used in the differentiation and clinical diagnosis of thyroid disease in particular hyperthyroidism. Approximately 5-10% of all cases of hyperthyroidism have elevated $T_3$ concentrations accompanied by normal $T_4$ concentration. Such clinical conditions make it vital to establish that $T_3$ levels are normal before excluding the diagnosis of hyperthyroidism. Serum $T_3$ level is also an excellent indicator for the ability of the thyroid to respond to stimulation and suppression test. Thyroxine or $T_4$ is the most commonly measured thyroid hormone for the diagnosis of thyroid function (Mansourian, 2011). Measurement of TSH using sensitive assay is presently the recommended initial screening test when thyroid disease is suspected (Mahajan and Singh, 2011). TSH is secreted from the anterior pituitary gland and induces the production of thyroxine ($T_4$) and triiodothyronine ($T_3$) from the thyroid gland (Sembulingam and Sembulingam, 2010).

### 3.1.3 Biochemical and Clinical Parameters Analyzed in the Selected Patients

After the diagnosis of thyroid disorder, 50 patients each of hypo and hyperthyroid patients were selected of which 25 were women and 25 were men and follow up was noted for one year. Patients diagnosed with thyroid disorder for the first time and who have not undergone treatment before were selected. The study group was above 40 years of age. The selected hypo and hyperthyroid patients were compared with age and sex matched control ($n=10$) who were not having thyroid problem. Serum thyroid hormones ($T_3$ and $T_4$), TSH, thyroid antibodies (TPO Ab and Tg Ab) and lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C) of the selected thyroid patients and control were analysed (table 2). Detection of autoantibodies to the two major thyroid antigens thyroglobulin and thyroid peroxidase is valuable in the diagnosis of patients with thyroid disease (Mazzaferri et al., 2003). The presence of anti-thyroglobulin autoantibodies has been shown to be a strong
indicator of chronic autoimmune thyroid diseases such as Hashimoto’s thyroiditis and Graves’ disease. While many patients elaborate to both thyroid antigens, several cases have been shown to be anti-thyroglobulin positive and anti-thyroid peroxidase negative or vice versa. Therefore, combined determination of both anti-thyroglobulin and anti-thyroid peroxidase antibodies might provide the most accurate diagnostic tool for thyroid autoimmunity (Spencer, 2010).

Table 2

Biochemical and clinical parameters analyzed in the selected thyroid patients

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Method of Analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triiodothyronine (T₃)</td>
<td>ELISA</td>
<td>Wisdom (1976)</td>
<td>III</td>
</tr>
<tr>
<td>2</td>
<td>Thyroxine (T₄)</td>
<td>ELISA</td>
<td>Wisdom (1976)</td>
<td>IV</td>
</tr>
<tr>
<td>3</td>
<td>Thyroid Stimulating Hormone (TSH)</td>
<td>ELISA</td>
<td>Soos and Siddle (1982)</td>
<td>V</td>
</tr>
<tr>
<td>4</td>
<td>Thyroid Peroxidase IgG Antibodies (TPO Ab)</td>
<td>ELISA</td>
<td>Davies and DeBernado (1983)</td>
<td>VI</td>
</tr>
<tr>
<td>5</td>
<td>Thyroglobulin IgG Antibodies (Tg Ab)</td>
<td>ELISA</td>
<td>Kuppers et al. (1993)</td>
<td>VII</td>
</tr>
<tr>
<td>6</td>
<td>Total cholesterol</td>
<td>CHOD-PAP Method</td>
<td>Allain et al. (1974)</td>
<td>VIII</td>
</tr>
<tr>
<td>7</td>
<td>Triglycerides</td>
<td>KIT METHOD</td>
<td>Schettler and Nussel (1975)</td>
<td>IX</td>
</tr>
<tr>
<td>8</td>
<td>HDL-C</td>
<td>KIT METHOD</td>
<td>Burstein et al.(1970)</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>LDL-C</td>
<td>KIT METHOD</td>
<td>Wiebe and Warnik (1994)</td>
<td>XI</td>
</tr>
<tr>
<td>10</td>
<td>VLDL-C</td>
<td>Calculation</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Thyroid hormones have their effect on lipid metabolism. Thyroid disorders are likely to increase the risk of cardiovascular disease by affecting the levels of cholesterol. Measurement of serum cholesterol levels are useful in
the evaluation of the risk of the coronary arterial occlusion, atherosclerosis, myocardial infarction, liver function, biliary function, intestinal absorption, thyroid function and adrenal disease (Mahajan and Singh, 2011). Measurement of triglyceride is important in the diagnosis and management of hyperlipidemias. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus and endocrine disturbances (Rizos et al., 2011).

Phase II

3.2 Phytochemical Analysis of the Selected Seaweed and the Plant Sample

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Natarajan et al., 2011). Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition which are found as secondary plant metabolites in one or more parts of these plants. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids and essential oils (Matkowski et al., 2008). Phytochemicals such as carotenoids, tocopherols, ascorbates and phenols present in plants are natural antioxidants and have an important role in health care systems (Lamaeswari and Ananthi, 2012).

Hence, in the present study the selected seaweed (brown algae) and the plant sample (Zea mays L.) were analyzed for the presence of phytochemicals.

3.2.1 Selection of Seaweed (brown algae) and Medicinal Plant (Zea mays L.) for the Study

Medicinal plants have a long history of use by human beings for the cure of various ailments (Sharma and Kumar, 2012). The thyroid hormone thyroxine is made up of iodine, which plays a major role in body metabolism. The seaweed and the plant sample selected for the treatment of hypo and hyperthyroidism that is brown algae and Zea mays L. are known for their iodine content.
Seaweeds are great potential producers of secondary metabolites which are not found in terrestrial environment. Marine algae are among the richest sources of known novel bioactive compounds (Lavanya and Veerappan, 2011). *Sargassum* a brown canopy forming macro-algae originated in Asia is considered as one of the most aggressive of the marine macro algal invaders (Bouderesque and Verlaque, 2002). *Sargassum* was used to prevent goiter in ancient China and contain various secondary metabolites with biological activities (Cadena, 2010).

Maize is one of the most important food crops. Presence of cyanogenic glucosides, which are antithyroidal substances in maize, is well established (Amar *et al*., 2009). Sweet corn is a popular crop and its plantation is increased considerably in many parts of the world in the last ten years (Voichita and Ioan, 2009). Traditionally corn silk has been used as diuretic, antilithiasic, uricosuric and antiseptic (Ebrahimzadeh *et al*., 2008). No scientific proof is available on the use of sweet corn silk for the treatment of hyperthyroidism. Therefore, the present study was carried out to find the antithyroid potential of sweet corn silk.

Hence, *Sargassum wightii* Greville and *Maydis stigma / Zea mays* hair (sweet corn silk) were selected for the study.

### 3.2.2 Collection and Preparation of the Selected Samples

The seaweed *Sargassum wightii* Greville was collected from Kanyakumari district. The whole algae were washed in sea water and fresh water thoroughly to remove the epiphytes and other contamination. Then the sample was immediately transferred into a polyethylene bag with a small hole to leak out seawater drops and shade dried. Fresh sweet corn was collected from Unicorn food product in Salem for the study. Corn silk was removed, washed and shade dried. Selected samples were authenticated by Botanical Survey of India, Coimbatore.

The shade dried *Sargassum wightii* Greville and *Maydis stigma* (sweet corn silk) were ground into coarse powder separately and stored in clean containers at room temperature for further studies.
3.2.3 Estimation of Iodine Content in the Study Samples

Iodine has long been known as an essential element for humans and for mammals. It is concentrated in the thyroid gland as the thyroid hormone, thyroxine (Fuge, 2007). Deficiency or excess of iodine leads to abnormal thyroid function. For normal thyroid function to be sustained appropriate iodine intake is required (Benbassat et al., 2004). Hence, it is essential to know the iodine content in the samples selected for the study. Dried powdered sample of *Sargassum wightii* Greville and *Maydis stigma* (sweet corn silk) were analyzed for its iodine content and the procedure in detail is given in appendix XII.

3.2.4 Qualitative Analysis for the Presence of Phytochemicals

3.2.4.1 Preliminary Phytochemical Screening of *Sargassum wightii* Greville and *Maydis stigma*

Fresh samples of *Sargassum wightii* Greville and *Maydis stigma* were screened for the presence of phytochemicals. Five grams of *Sargassum wightii* Greville and *Maydis stigma* were weighed, mashed and homogenized with 50ml of alcohol, acid (1% HCl) and water separately. These were boiled for one hour, cooled, filtered and used for the analysis of phytochemicals. The extract was analyzed for the presence of phytochemicals such as flavonoids, phenols, anthocyanins, tannins, saponins, steroids, alkaloids and terpenoids using standard procedure. The detail procedure is given in appendix XIII.

3.2.4.2 Preparation of the Organic Extract and Phytochemical Analysis of Selected Samples

Separately dried powder of *Sargassum wightii* Greville and *Maydis stigma* were successively extracted with different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, methanol and ethanol with their increasing order of polarity by soxhlation for 6-12 hours. For the extraction 20 g of dried powdered sample was used with 200 ml of the solvent. Then the extract obtained were collected separately and kept for further analysis. The qualitative phytochemical tests of various extracts of *Sargassum wightii* Greville and
Maydis stigma were carried out using standard procedure. The procedure in detail is given in appendix XIII.

3.2.5 Determination of Free Radical Scavenging Activity (DPPH Radical Scavenging Activity) in the Selected Samples

The stable 2, 2-dipheny-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extract. Different concentrations (10-100 µg) of various extracts (aqueous, benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether) of Sargassum wightii Greville and Maydis stigma were added with an equal volume of methanolic DPPH solution (0.5 mM) and incubated at 37°C for 15 min separately. The absorbance was recorded at 517 nm. The detailed procedure is given in appendix XIV. IC\textsubscript{50} values denote the concentration of sample, which is required to scavenge 50 per cent of DPPH free radicals.

3.2.6 Evaluation of the Total Antioxidant Activity in the Selected Samples

The phosphomolybdenenum method was used to evaluate the total antioxidant activity of the various extracts of Sargassum wightii Greville and Maydis stigma. Antioxidants can reduce Mo (IV) to Mo (V) and the green phosphate / Mo (V) compounds, which have an absorption peak at 695 nm, were generated subsequently. The detailed procedure of which is given in appendix XV.

3.2.7 Characterization of Bioactive Component Present in the Study Samples using HPLC, HPTLC, GC-MS, UV-Visible and FT-IR Analysis

3.2.7.1 Characterization of Phytochemical Constituents by HPLC Analysis

The HPLC analysis of methanolic extracts of Sargassum wightii Greville and Maydis stigma was performed using Schimatdzu HPLC system with UV-Vis detector (Model hpv20 A). The separation was performed on a Luna C18 column (2.0 mm i.d. X 150 mm, 3 µm particle size) with guard column (2.0 mm i.d. X 40 mm, 3 µm). After the optimization of conditions, mobile phase
Experimental Procedure

composed of deionized water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) was used. Sample injection volume was 20 µl and UV absorbance was monitored at 340 nm.

3.2.7.2 Characterization of Phytochemical Constituents by HPTLC Analysis

A densitometric HPTLC analysis of *Sargassum wightii* Greville and *Maydis stigma* was performed for the development of characteristic fingerprint for alkaloid, flavonoid, glycoside, phenolic and steroid profile. Methanolic extract of *Sargassum wightii* Greville and *Maydis stigma* was centrifuged at 3000 rpm for 5 min. Supernatant was used as test solution for HPTLC analysis. 2 µl of test solution and 2 µl of standard solution was loaded as 5 mm band length in the 3 x 10 Silica gel 60F<sub>254</sub> TLC plate for alkaloid and glycoside profile whereas for flavonoid, phenol and steroid profile, samples were applied as 6 mm wide band in the 3 x 10 Silica gel 60F<sub>254</sub> TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV 366 nm. The developed plate was sprayed with respective spray reagent and dried at 100 °C in Hot air oven.

The plate was photo-documented in Day light and UV 366 nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm for alkaloid, flavonoid and phenolic profile while for glycoside and steroid profile scanning was done at 500 nm. The peak table and peak densitogram for each profile were noted. The software used was winCATS 1.3.4 version. Standard, mobile phase and spray reagent used for the HPTLC analysis of each profile is as follows:
Experimental Procedure

ALKALOID PROFILE

Alkaloid standard- Colchicine was used as reference marker.

Mobile phase

Ethyl acetate-Methanol-Water (10: 1.35: 1)

Spray reagent

Dragendorff’s reagent followed by 10% ethanolic sulphuric acid reagent.

FLAVONOID PROFILE

Flavonoid standard- Quercetin used as reference marker.

Mobile phase

Chloroform-Ethyl acetate-Glacial acetic acid (60: 35: 5)

Spray reagent

1% Ethanolic Aluminium chloride reagent.

GLYCOSIDE PROFILE

Glycoside standard- Stevioside used as reference marker.

Mobile phase

Ethyl acetate-Ethanol-Water (8: 2: 1.2)

Spray reagent

Libermann-Burchard reagent.

PHENOLIC PROFILE

Phenolic standard- Catechol was used as reference marker.

Mobile phase

Toluene-Acetone-Formic acid (4.5: 4.5: 1)

Spray reagent

20% Sodium carbonate solution followed by Folin Cio-calteu reagent.

STEROID PROFILE

Steroid standard- Stigmasterol was used as reference marker.

Mobile phase

Toluene-Acetone (9:1)
Experimental Procedure

Spray reagent
Anisaldehyde sulphuric acid reagent.

3.2.7.3 Characterization of Phytochemical Constituents by GC-MS Analysis

The GC-MS analysis of methanolic extract of *Sargassum wightii* Greville and *Maydis stigma* was performed with Thermo Gc-Trace Ultra Ver: 5.0, Thermo MS DSQ II GC-MS instrument equipped with TR 5-MS capillary standard non-polar column of 30 m length, 0.25 mm i.d. and 0.25 µm thicknesses. Helium was the carrier gas used at a flow rate of 1ml/min. Initial column temperature was set at 50°C for 3 min then the temperature was increased by 7°C/min to reach 250°C and the sample Injector temperature was maintained at 250°C throughout the experimental period.

The injection volume was 1µl. The mass spectroscopic analysis was done with 70 eV electron energy level, between 50 m/z and 500 m/z for the duration of 38 min. GC-MS chromatograms were analyzed using Xcalibur, version 1.4 software integrating each peak individually.

3.2.7.4 Characterization of Phytochemical Constituents by UV-Visible Spectrum Study

Non linear optical property of the selected samples has been tested by Kurtz powder technique. Its optical behaviour was examined by Ultraviolet-Vis spectrophotometer instrument model Ultraviolet-Vis 1700 and found that the crystal is transparent in the region between 200-800 nm.

3.2.7.5 Characterization of Phytochemical Constituents by FT-IR Analysis

The FT-IR analysis of powder samples was carried out by using Schimatdzu 8400 model FT-IR and FT Raman Spectrometer using KBr pellet and powder form respectively. The FT-IR was recorded in the range 400-4000 cm\(^{-1}\) for both the samples. The assignments were made assuming Cs point group symmetry. The various modes of vibration were identified and assigned.


### Phase III

#### 3.3 Animals studies

To find out the effect of methanolic extract of *Sargassum wightii* Greville and *Maydis stigma* on experimental animals, *in vivo* studies were carried out.

##### 3.3.1 Selection and Maintenance of the Animals

33 male albino rats weighing 150-200 g were selected for the study. They were brought from Central Animal House (Kerala). The rats were maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark/light cycle (14/10 hr). The animals were kept in neat cages, lined with husk and fed with standard pellet diet and water *ad libitum*. The rats were acclimatized to laboratory conditions for 15 days before the commencement of the experiments. University Animals Ethical Committee (Reg no: 623/02/b/CPCSEA) has reviewed and approved all procedures described.

##### 3.3.2 Grouping of the Animals

Animals were grouped separately for hypo and hyperthyroid experiment, each group consisting of three animals. The groups are as follows in table 3 and 4. Control group is same for both the hypo and hyperthyroid experiments.

##### 3.3.3 Weight of the Experimental Animals

Body weights of the experimental animals were noted before and during the experiment.

##### 3.3.4 Biochemical Parameters Analyzed in Experimental Animals

Blood was collected from experimental rats after overnight fasting. It was collected from orbital sinus using anesthesia (diethyl ether) (Hoff, 2000). Serum was separated by centrifugation and analyzed for the following parameters:

##### 3.3.4.1 Thyroid Hormone Level

Thyroid hormones namely triiodothyronine ($T_3$), thyroxine ($T_4$) and thyroid stimulating hormone were analyzed in the serum of experimental rats.
Experimental Procedure

Plate I
Experimental animal

Oral feeding

Retro-orbital plexus- Blood collection
### Table 3
**Grouping of the experimental rats (Hypothyroid experiment)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>Control- Normal Diet</td>
</tr>
<tr>
<td>GROUP II</td>
<td>Hypothyroid induced rats (using methimazole-0.04 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP III</td>
<td>Hypothyroid induced rats treated with standard drug thyroxine(0.2 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>Hypothyroid induced rats treated with methanolic extract of <em>Sargassum wightii</em> Greville (200 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP V</td>
<td>Hypothyroid induced rats treated with methanolic extract of <em>Sargassum wightii</em> Greville (300 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>Hypothyroid induced rats treated with methanolic extract of <em>Sargassum wightii</em> Greville (400 mg/kg body weight for 21 days)</td>
</tr>
</tbody>
</table>

### Table 4
**Grouping of the experimental rats (Hyperthyroid experiment)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>Control- Normal Diet</td>
</tr>
<tr>
<td>GROUP II</td>
<td>Hyperthyroid induced rats (using thyroxine-0.2 mg /kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP III</td>
<td>Hyperthyroid induced rats treated with standard drug methimazole (0.04 mg /kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>Hyperthyroid induced rats treated with methanolic extract of <em>Maydis stigma</em> (200 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP V</td>
<td>Hyperthyroid induced rats treated with methanolic extract of <em>Maydis stigma</em> (300 mg/kg body weight for 21 days)</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>Hyperthyroid induced rats treated with methanolic extract of <em>Maydis stigma</em> (400 mg/kg body weight for 21 days)</td>
</tr>
</tbody>
</table>
Experimental Procedure

Triiodothyronine (T<sub>3</sub>)

Triiodothyronine (T<sub>3</sub>) present in the experimental rat’s serum was analyzed by Enzyme-Immuno assay (Wisdom, 1976). The procedure is given in detailed in appendix III.

Thyroxine (T<sub>4</sub>)

Triiodothyronine (T<sub>4</sub>) present in the experimental rat’s serum was analyzed by Enzyme-Immuno assay (Wisdom, 1976). The detail procedure is given in appendix IV.

Thyroid Stimulating Hormone (TSH)

Thyroid stimulating hormone (TSH) in the serum of experimental rats was analyzed by Enzyme-Immuno assay (Soos and Siddle, 1982). The detail procedure is given in appendix V.

3.3.4.2 Lipid Profile

The lipid profile namely total cholesterol (TC), triglycerides (TGs), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and very low density lipoprotein cholesterol (VLDL-C) were estimated in the serum collected from experimental rats.

Estimation of Total Cholesterol (TC)

Total cholesterol present in the serum of experimental rats was estimated by CHOD-PAP method (Allain et al., 1974) as given in appendix VIII.

Estimation of Triglycerides (TGs)

Triglycerides in the serum of experimental rats were estimated by kit method (Schettler and Nussel, 1975). The detail procedure is given in appendix IX.
Experimental Procedure

Estimation of High Density Lipoprotein Cholesterol (HDL-C)

High Density Lipoprotein cholesterol (HDL-C) in the serum of experimental rats was determined by kit method (Burstein et al., 1970) as given in appendix X.

Estimation of Low Density Lipoprotein Cholesterol (LDL-C)

Low Density Lipoprotein cholesterol (LDL-C) in the serum of experimental rats was determined by kit method (Wiebe and Warnik, 1994). The detailed procedure is given in appendix XI.

Estimation of Very Low Density Lipoprotein cholesterol (VLDL-C)

Very Low Density Lipoprotein cholesterol (VLDL-C) value was determined in the serum of experimental rats by using the formula,

\[
\text{VLDL-C (mg/dl)} = \frac{\text{Triglycerides}}{5}
\]

3.3.5 Induction of Experimental Hypo and Hyperthyroidism

Experimental hypothyroidism was induced to Group II, III, IV, V and VI rats by using methimazole (0.04 mg/kg body weight) orally for 21 days for hypothyroid experiment. Hyperthyroidism was induced in Group II, III, IV, V and VI rats using thyroxine (0.2 mg/kg body weight) orally for 21 days for hyperthyroid experiment. Induction of both the conditions was confirmed by analyzing the serum thyroid hormone level.

3.3.6 Treatment of the Animals with Standard Drug

Hypothyroid induced group III experimental rats were treated using standard drug thyroxine at a concentration of 0.02 mg/kg body weight and were given orally for a period of twenty one days (Chakrabarti et al., 2007). While hyperthyroid induced group III rats were treated with standard drug methimazole at 0.04 mg/kg body weight orally for twenty one days (Isman et al., 2003).
3.3.7 Treatment of the Animals with Sample Extracts

Hypothyroid induced group IV, V and VI animals were treated with 200, 300 and 400 mg/kg body weight of methanolic extract of *Sargassum wightii* Greville orally for twenty one days.

Similarly, hyperthyroid induced group IV, V and VI animals were treated with 200, 300 and 400 mg/kg body weight of methanolic extract of *Maydis stigma* orally for twenty one days respectively.

3.3.8 Histopathological Studies

At the end of the experiments animals were scarified, the thyroid glands of rats were dissected out. Wet weight of thyroid gland was taken and gland was fixed in formal buffer solution and then embedded in paraffin after usual processing. Sections were cut at 5-6 µm thickness stained and viewed under a microscope. The detailed procedure is given in appendix XVI.

3.4 Statistical Analysis

Results are expressed as the mean ± SD. Statistical significance was evaluated by One way analysis of variance (ANOVA) using SPSS version (17.0) and the individual comparisons were obtained by the Duncan's multiple range test (DMRT) (Duncan, 1957). A value of p<0.05 was considered to indicate a significant difference between groups. For biochemical parameters comparison was made using student “t” test (p <0.05).