The methodology of the study pertaining to “Nutrient Composition, Antioxidant Activity and Therapeutic Use of Selected Seaweeds”, are presented under the following headings.

3.1 Phase I
   3.1.1 Selection and study of morphological characteristics of selected seaweeds
   3.1.2 Analysis of nutrient composition of the selected seaweeds
   3.1.3 Assessment of the microbial content of the selected seaweeds

3.2 Phase II
   3.2.1 Toxicological studies of selected seaweeds
   3.2.2 Estimation of antioxidant and antimicrobial activity of the selected green seaweeds

3.3 Phase III
   3.3.1 Product development and acceptability of value added products with green seaweeds

3.4 Phase IV
   3.4.1 Estimation of nutrient content and \textit{invitro} iron bioavailability of the value added seaweed chocolate
   3.4.2 Study the impact of seaweed chocolate among anaemic adolescent girls

3.5 Phase V
   3.5.1 Assessment of phytonutrient content and \textit{invivo} antioxidant activity of value added seaweed tea.
   3.5.2 Study the impact of seaweed tea among adult male subjects with precancerous oral lesions.
3.1 PHASE I

3.1.1 Selection and study of morphological characteristics of selected seaweeds

Seaweeds are highly concentrated in the coastal belt of Gulf of Mannar, Rameswaram to Kanniyakumari in Tamilnadu (Figure1). The data indicates that a total of 302 species of seaweeds are available in this belt and more than 263 species are edible.

Pamban and Thonithurai are two important coastal zones of Gulf of Mannar where seaweeds are abundantly available and cultivated. With the guidance from the scientists of Marine Algal Research Station and Central Marine Fisheries Research Institute (Mandapam) the following four seaweeds were identified: 1) brown (*Padina tetrastomatica*), 2) red (*Acanthophora spicifera*) 3) and 4) green (*Ulva lactuca* and *Ulva reticulata*) for the study. The selected seaweed species are edible seaweeds consumed occasionally by the local population.

Seaweeds were collected fresh with the help of sea divers trained in handpicking these seaweeds. The collected seaweeds were rinsed first in sea water in the collected area and packed in aseptic bags. They were further cleaned with fresh water to remove extraneous matter such as epiphytes, sand particles, pebbles and shells. Plate I shows the collection of seaweeds.

Further, these seaweeds were washed thoroughly to ensure that all the dirt was removed and spread out in room temperature for drying for a period of 24-32 hours. The shade dried samples were ground to fine powder and stored in air tight containers for use (Plate II). The morphological characteristics namely type, colour, texture, shape and habitat of the collected seaweeds were observed and the taxonomical classification of the selected seaweed species was carried out according to Fritsch, (1935).
FIGURE 3
COAST LINE OF TAMILNADU - RAMESWARAM
PLATE I
COLLECTION OF SEAWEEDS

PLATE II
DRY SEAWEED POWDERS

*Ulva reticulata*

*Ulva lactuca*
### 3.1.2 Analysis of nutrient composition of the selected seaweeds

The nutrient content of all the four seaweeds were analyzed using standard procedures (Appendix I) for various macro and micro nutrients and as well as heavy metals (Table 1). The nutrient content of the seaweeds was analysed in order to understand the safety of the seaweeds to be used as raw or in a semi processed form in the formulation of seaweed value added food products. The energy value was computed from the protein, lipid and carbohydrate content of the selected seaweeds.

#### TABLE 1
**METHODS USED FOR ANALYSIS OF NUTRIENT CONTENT OF SEAWEEDS**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macro nutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Anthrone method</td>
<td>Hedge <em>et al.</em> (1962)</td>
</tr>
<tr>
<td>Protein</td>
<td>Spectrophotometry</td>
<td>Lowry <em>et al.</em> (1951) UV</td>
</tr>
<tr>
<td>Lipid</td>
<td>-</td>
<td>Freeman <em>et al.</em> (1957)</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>Acid Alkali Digestion</td>
<td>Raghuramulu <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><strong>Micro nutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>Spectrophotometry</td>
<td>Hawk <em>et al.</em> (1957)</td>
</tr>
<tr>
<td>magnesium</td>
<td>Spectrophotometry</td>
<td></td>
</tr>
<tr>
<td>Beta carotene</td>
<td>Dye reduction</td>
<td>Ranganna (1976)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>Sadasivam <em>et al.</em> (1987)</td>
</tr>
<tr>
<td><strong>Heavy metals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cadmium</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bio active compounds</strong></td>
<td>Gas chromatography</td>
<td>Clarus 500 Perkin Elmer model</td>
</tr>
</tbody>
</table>
3.1.3 Assessment of the microbial content of the selected seaweeds

To assess the moisture, total bacterial count and type of common microorganisms found, the four seaweeds were subjected to microbial analysis initially before they were converted to powder form. The total bacterial count was carried out using streak plate method and microbial tests were done to find out the organisms namely *Escherichia coli, Salmonella, Bacillus* and *Pseudomonas*. The tests were also carried out using the dry powders on the 90th day of storage. The procedure used is given in Appendix II.

3.2 PHASE II

3.2.1 Toxicological studies of selected seaweeds

The four seaweeds collected for the study namely *Ulva lactuca, Ulva reticulata, Acanthophora spicefera and Padina tetrastomatica* were subjected to acute toxicity and sub acute toxicity to select the minimum lethal dose and to rule out the effect of any toxins.

The tests were carried out based on the Organisation For Economic Co-Operation and Development (OECD) guideline for testing of chemicals 410.423 (2001).

3.2.1.1 Acute toxicity

2000 and 5000mg of seaweed powders of all the four selected seaweeds were separately well macerated in a mortar and pestle and a suspension was made in one per cent carboxy methyl cellulose. Seaweed test sample was prepared fresh prior to each dosing.

The dosing started with 2000 mg/kg weight of the rat and tested with additional increment of 300 mg and increased to 5000 mg. If the rat died in any one dosing the next low level dose of the seaweed was administered to the rats.
**Procedure for study**

Four groups of eight week old healthy female rats weighing (110-150g) were taken for the study. A total of twenty eight rats (7 rats in a group) were used for the administration of the four seaweeds with the test compound at a dose level from 2000-5000 mg/kg body weight after 18 hours of starvation. Initially one rat in the group was dosed with 2000mg/kg body weight and observed for clinical signs and the dose was increased up to 5000mg/kg body weight and the rat was observed again. If the rat is alive then the same dose was administered to the other two rats in the group and observed for clinical symptoms.

**Animal house condition**

Animal house temperature was maintained between 19 - 25°C and humidity 30 –70 per cent. Temperature and humidity was measured daily. The facility was provided with 12 hour light and 12 hour darkness. Standard poly propylene rat cages with stainless steel top grill were used to house the animals. The cages were autoclaved. Cleaned paddy husk was used as a bedding material. Animals were housed in cages containing not more than two per cage. Food and water were supplied to the animals. Standard rat pellet feed supplied by Lipton India Limited, Bangalore and filtered water was given to the rats. Both drinking water and feed were provided adlibitum except during pre dose fasting where only water was provided. Plate III shows the housing and feeding of rats.

**Animal observation**

Single dose of 2000mg/kg or 5000mg/kg body weight was administered to the animals and observed on the seventh and 14th day. After administration, body weight, gross pathological examination, weight of liver and kidney were noted on the 14th day after necroscopy of the animal. Body weight for all the 14 days was observed. Behaviour of the animal was also noted from 30 minutes after dosing and thereafter, observed after 1,2,4 and 6 hour after dosing. Thereafter the animals were observed once every 24 hours for 14 days.
From the results of the study only two species of green algae were safe at a level of 5000 mg/kg body weight and these seaweeds were subjected to sub acute toxicity studies.
3.2.1.2 Sub acute toxicity

Animal chosen for the study

Adult male albino rats of wistar strain weighing around 250-300g were obtained from the animal house of the Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of 25 ± 2°C and 55 ± 65 per cent relative humidity. A 12±1°C was maintained in the animal house till the animals adjusted to the laboratory conditions and were fed with commercially available rat chow (Hindustan Lever Limited, Bangalore, India) and had free access to water. The animals were divided into three groups, each group containing 6 animals. The animals were supplemented with a single dose of 5000mg/day of seaweeds orally for a period of 60 days.

The groups are as follows:

Group I : Vehicle control (distilled water)
Group II : Administered seaweed Ulva lactuca
Group III: Administered seaweed Ulva reticulata

Physical changes

Morphological changes in the body of the treated animals were noticed everyday by careful visual observation to see any physical changes in the body like hair fall, necrosis, infection, overgrowth and overall activeness of the animals. The control group and experimental group animals were weighed every morning with the use of a balance. The changes in the body weight were noted. The feed consumed, water intake and excreta quantity of all the group animals were measured everyday by standard techniques using measuring cylinder and balance (Sartorius).
Biochemical analysis

The major hematological and biochemical parameters, mineral and enzymes were analysed. The biochemical tests were carried out using the serum sample. The details are given in Table 2.

**TABLE 2**
METHODS USED FOR ANALYSIS OF BIOCHEMICAL PARAMETERS

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Biochemical parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological parameters</td>
<td>Haemoglobin (g/dl) Cyanmethhemoglobin method Ferritin (mg/dl), iron (mcg/dl), red blood cells (x10^{6} µl), platelets (x 10^{4} µl), white blood cells (x10^{3} µl), neutrophils (x10µl), lymphocytes (x10µl)</td>
<td>(Sood, 1990 and Raghuramalu et al., 2003)</td>
</tr>
<tr>
<td>Minerals</td>
<td>Sodium (mEq/L), potassium (mEq/L), chloride (mEq/L), phosphorus (mg/dl), calcium (mg/dl) and magnesium (mg/dl)</td>
<td>(Raghuramalu et al., 2003)</td>
</tr>
<tr>
<td>Biochemical parameters</td>
<td>Glucose (mg/dl), cholesterol (mg/dl), total protein (g/dl), urea nitrogen (mg/dl), albumin (g/dl), globulin (g/dl)</td>
<td>(Raghuramalu et al., 2003)</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Alanine aminotransferase (u/l), Aspartate aminotransferase (u/l) and Alkaline phosphatase (u/l)</td>
<td>(Raghuramalu et al., 2003)</td>
</tr>
</tbody>
</table>

The experiments were designed and conducted in accordance with the Institutional Animal Ethics Committee Number 1282/ac/09/CPCSEA. The procedures used are given in Appendix III.
3.2.2 Estimation of antioxidant and antimicrobial activity of selected green seaweeds

3.2.2.1 Antioxidant activity of *Ulva lactuca* and *Ulva reticulata*

The antioxidant and antimicrobial activities of the two seaweeds (*Ulva lactuca* and *Ulva reticulata*) alone was studied. Based on the results obtained from the toxicological evaluation, *Ulva lactuca* and *Ulva reticulata* were safe for rats and further biological evaluation was carried out only for the above mentioned two green seaweeds. *In vitro* antioxidant activity using 1, 1 diphenyl 2, picryl hydrazyl (DPPH) radical scavenging method was carried out. Radical scavenging activity was determined by spectrophotometric method based on the reduction of a methanol solution of DPPH using the method of Blois (1958). One milliliter of various concentrations of the extract was added to 1 ml of a 0.004 per cent methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 minutes in the dark. Then the absorbance was measured at 517 nm against a blank by a spectrophotometer.

Inhibition of free radical DPPH in per cent was calculated according to formula:

\[
\text{Per cent} = \frac{(A \text{ blank} \times A \text{ sample})}{A \text{ blank}} \times 100
\]

A blank is the absorbance of the control reaction (containing all reagents expect the test compound) and A sample is the absorbance of the test compound. Extract concentration providing 50 per cent (IC50) was calculated from the graph plotting inhibition per centage against seaweed extract concentration. The tests were carried out in triplicate and Butylated Hydroxy Toluene (BHT) was used as control.

3.2.2.2 Antimicrobial activity of *Ulva lactuca* and *Ulva reticulata*

To find the possibility of incorporating seaweeds in food for the development of value added products the inhibitory activity of the seaweed for bacteria and fungi was carried out. Antibacterial analysis was conducted using Standard Agar Well Diffusion method (Perez *et al.*, 1990; Erdemoglu *et al.*, 2003;
Bagamboula et al., 2004). Antibacterial tests were evaluated by measuring the zone of inhibition against the test microorganisms namely *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Vibrio cholera* and *Pseudomonas mirabilis*. Antifungal tests were conducted for *Candida albicans* and *Candida tropicalis*. These microorganisms were tested as they were commonly found in food. Ethanol and methanol were used as solvent control. Chloramphenicol (100 µg) disc was used as reference antibacterial agent and miconazole (100µg) was used as reference for antifungal activity. The tests were carried out in triplicates.

The Minimal Inhibitory Concentration (MIC) was estimated using the Broth Dilution Method (Van der Berghe and Vlietinck, 1991) for the above microorganisms. Dilutions of extract from 0.075 to 2.0 mg/mL were used. Test bacterial culture was used at the concentration of $10^5$ colony forming units/ml. The lowest seaweed extract concentration that prevented visible bacterial growth after 24 hour of incubation at 37°C was taken as Minimal Inhibitory Concentration values. Experiments were triplicated. The detailed procedure is given in Appendix IV.

### 3.3 PHASE III

#### 3.3.1 Product development and acceptability of value added products with green seaweeds

##### 3.3.1.1 Formulation of product

Value added products were developed with the incorporation of seaweed *Ulva lactuca* and *Ulva reticulata* so that they can be used to supplement humans and determine the therapeutic impact. The impact of the seaweeds was studied for anaemia and condition of precancerous oral lesion. Anaemia was studied because of its high prevalence among adolescent population. In the area selected for the study fishermen population was high and as these men used tobacco very commonly the chances of development of oral cancer were high and hence precancerous oral
lesion condition was selected for the study. Ethical clearance from the Government Hospital, Madurai, Tamil Nadu was obtained and the clearance letter is given in Appendix V.

Seven recipes each incorporating *Ulva lactuca* and *Ulva reticulata* were developed. The products prepared were tomato soup, vegetable soup, tomato spread, seaweed tea, chocolate, nutrient ball and bun. The selection of the products was based on the ease in preparation and packaging.

### 3.3.1.2 Standardization of the recipe

Standardization of recipes is a formula specifying the quality of each ingredient required to produce a specific quantity and quality of a particular food item (Khan, 1987).

A written set of description was followed for each recipe. Each ingredient was weighed using a weighing scale before and after preparation. Portion size and duration of preparation were noted in each case. All the recipes were standardized for one serving and repeated thrice to get consistent results. The recipes were then subjected to acceptability tests. Plate IV and Table 3 shows the standardized recipe for chocolate along with the ingredients used for preparation. The details of the standardized recipes are given in Appendix VI.

The acceptability test was rated by one hundred female students of a private college, Kilakarai, Ramanathapuram District. The selected subjects were asked to score the product using a score card. A score card is defined by Potter and Hotchkiss (2002) as an evaluation card, sample coded with letters or numbers with descriptive terms such as excellent, very good, good, fair and poor. The attributes scored were appearance, colour, texture/consistency, flavor and taste. A maximum score of five was given for each attribute. The product with the highest score for overall acceptability was taken for supplementation. Among the seven recipes
selected, tea and chocolate obtained maximum overall acceptability and hence were selected for supplementation. Plate V and Table 4 shows the standardized recipe and ingredients used for preparation of seaweed tea.

PLATE IV
INGREDIENTS USED FOR PREPARATION OF SEAWEED CHOCOLATE WITH *Ulva reticulata*

The seaweed was incorporated in green tea because the taste and flavor blended well with green tea than the regular black tea infusion.
TABLE 3
INGREDIENTS USED FOR VALUE ADDED SEAWEED CHOCOLATE AND STANDARD

<table>
<thead>
<tr>
<th>Ingredients (gm)</th>
<th>Seaweed chocolate (20g)</th>
<th>Standard (15g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry seaweed powder (<em>Ulva reticulata</em>)</td>
<td>5</td>
<td>Nil</td>
</tr>
<tr>
<td>Dark chocolate</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cashew nuts and almond</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bajra, Roasted bengal gram, Rice flakes, Green gram</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

PLATE V
INGREDIENTS USED FOR PREPARATION OF SEAWEED TEA WITH *Ulva lactuca*
### TABLE 4

**INGREDIENTS USED FOR VALUE ADDED SEAWEED TEA AND STANDARD**

<table>
<thead>
<tr>
<th>Ingredients (gm)</th>
<th>Seaweed tea (100ml)</th>
<th>Standard (100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry seaweed powder (<em>Ulva lactuca</em>)</td>
<td>5</td>
<td>Nil</td>
</tr>
<tr>
<td>Black tea powder</td>
<td>nil</td>
<td>5</td>
</tr>
<tr>
<td>Green tea powder</td>
<td>2.5</td>
<td>Nil</td>
</tr>
<tr>
<td>Ginger</td>
<td>2.5</td>
<td>2</td>
</tr>
<tr>
<td>Cardamom</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Palm candy</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Seaweed and standard tea was boiled for 15 minutes to reduce the volume to 100ml

#### 3.4 PHASE IV

**3.4.1 Estimation of nutrient content and *in vitro* iron bioavailability of the value added seaweed chocolate**

The developed seaweed chocolate was analysed for nutrients namely carbohydrate, protein, lipid and iron using standard procedures given in Appendix I. The energy values were computed using the nutritive value of Indian foods (Gopalan *et al*., 2007). The *in vitro* iron bioavailability (Miller *et al*, 1987) was estimated for *Ulva reticulata* extract, plain chocolate without seaweed and *Ulva reticulata* incorporated chocolate. This was estimated by dividing dialyzable iron with total iron of the product and expressed as available iron. The procedure used is given in Appendix VII. *In vitro* iron bioavailability was carried out to understand the percentage of available iron from seaweed since the selected seaweed had a considerably high iron content than any commonly used land vegetables.
3.4.2 Study the impact of seaweed chocolate among anaemic adolescent girls

According to Nadeem (2010) 60-70 per cent of the Indian adolescent girls are suffering from anaemia and hence to combat this nutritional deficiency seaweed chocolate was given as a nutritional supplement to adolescent girls since seaweed has high iron content.

3.4.2.1 Selection of subjects

A random population of 500 adolescent girls in the age group of 15-18 years studying in a private college at Kilakarai, Ramanathapuram district was selected to screen for anaemia. The classification given by World Health Organisation (2000) was used to categorize the mild, moderate and severe anaemic students. From those students who were moderately anaemic, 100 adolescent girls were selected by purposive sampling. Table 5 gives the classification of anemia as per World Health Organisation (WHO).

The one hundred adolescent girls were divided into 50 each with one group serving as the control and the other as the experimental.

<table>
<thead>
<tr>
<th>Classification of Anaemia</th>
<th>Heamoglobin Level (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Moderate</td>
<td>7-9</td>
</tr>
<tr>
<td>Mild</td>
<td>9-11</td>
</tr>
</tbody>
</table>

3.4.2.2 Socio-economic status of the anaemic adolescent subjects

An Interview schedule (Appendix VIII) was formulated to collect information regarding the socio economic status, age, education and occupational status and income of the head of the family.
3.4.2.3 Assessment of nutritional status

Anthropometric measurements namely height and weight were recorded for all the one hundred anaemic adolescents girls. Body mass index (BMI) was calculated by using the formula:

\[ \text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}} \]

The values were compared with standard per centile chart given by Center for Disease Control, (2000) (Appendix IX).

3.4.2.4 Dietary habits

Data regarding the nature and quantities of foods consumed were collected using a 24 hour recall survey. The 24 hour recall method of data collection requires individuals to remember the specific foods and units of foods they consumed in the past 24 hours (Mahan and Stump, 2008). The raw equivalents was calculated and the nutrients namely energy, carbohydrate, protein, fat, iron, beta carotene and vitamin C was computed by using the Food Composition Table from Nutritive Value of Indian foods given by ICMR (Gopalan, 2007). All the selected 100 adolescent girls were residing in the hostel and followed the same dietary pattern and hence the subjects were on controlled diet.

3.4.2.5 Clinical assessment

A clinical assessment schedule was used to elicit details on clinical signs like dry hair, pale pallor, dryness of mouth and nail pallor. With the help of a medical officer the clinical signs were determined for the 100 adolescent girls.

3.4.2.6 Study impact of the seaweed chocolate among anaemic adolescent subjects

The experimental group received 20 gm of the seaweed chocolate consisting of Ulva reticulata daily for a period of 120 days. The seaweed Ulva reticulata was found to be rich in iron content with 56 mg/100gm and hence was selected for use.
as a supplement for anaemic adolescent girls. The control group received 15gm chocolate without the seaweed. Before supplementation all the subjects were dewormed and biochemical analysis was carried out. Haemoglobin test was conducted for all the subjects on the initial, 30th and 120th day. The haemoglobin analysis on the 30th day was carried out in order to find, whether there were any adverse changes in the haemoglobin levels due to supplementation with seaweed chocolate. After the 30th day the supplementation was continued for further 90 days since it did not produce any change.

For all the selected adolescent girls, selected biochemical parameters were analysed before and after supplementation with seaweed chocolate. Haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin, red blood cells, white blood cells, total iron binding capacity, serum iron and serum ferritin were assessed using standard procedures (Appendix III).

3.5 PHASE V

3.5.1 Assessment of phytonutrient content and invivo antioxidant activity of value added seaweed tea

Before the seaweed tea was administered to the subjects, the phytonutrients namely polyphenol, chlorophyll, tannins, and beta carotene were analysed using standard procedures given in Appendix X. Seaweed tea incorporated with Ulva lactuca was selected for administration to adults with precancerous oral lesions because the results reported remarkable invitro antioxidant activity.

To confirm the results, invitro antioxidant activity was also determined. Rats were randomly divided into six groups of six animals each. Animals in first group served as control and received saline 1ml/100g vehicle, the second group received Vitamin C at 0.3mg/100g body weight and was used as standard. Groups A1, A2, A3, A4 received Ulva lactuca incorporated seaweed green tea orally at 0.5, 1.0, 1.5 and 2.0 ml/100g respectively for 20 days. After a period of 20 days the blood from the animals were drawn and tested for catalase (Abei, 1974), glutathione
3.5.2 Study the impact of seaweed tea on adult males with precancerous oral lesions

3.5.2.1 Selection of subjects

A precancerous lesion is “A morphologically altered tissue in which oral cancer is more likely to occur than its apparently normal counterpart” Ries et al. (1991). In order to identify the adults with precancerous oral lesions a community health camp was organized in a Private Medical Centre, Kilakarai and the patients were reviewed by an oncologist. Among the 650 beneficiaries 40 male subjects showed signs of dysplasia, an abnormal development or growth of tissues, organs, or cells (Kaugars, 1998). From the forty, thirty subjects who were willing to participate in the study were selected. Adult males with precancerous oral lesions in the age group of 35-45 years were divided into two groups with 15 as experimental and 15 as control and were selected for the study.

3.5.2.2 Socio economic status of the selected cancer subjects

An interview schedule is a written list of questions, open ended or closed ended, prepared for use by an interviewer in a person to person interaction (Kothari, 2005).

An interview schedule (Appendix XII) was formulated to collect the information regarding the socio economic status namely age, type of family, education, occupation and monthly income of the male subjects.

3.5.2.3 Assessment of nutritional status

The nutritional status was assessed through anthropometry, dietary habits and clinical assessment for the 30 male subjects with oral precancerous lesions.

Anthropometric measurements namely height and weight were recorded and Body Mass Index was calculated using their corresponding height and weight.
The BMI were compared with the standard values of International Obesity Task Force (IOTF, 2005) given in Appendix XIII.

3.5.2.4 Dietary habits

The information regarding dietary pattern was collected, using 24 hour diet recall method. The raw equivalents were calculated and the nutrient intake of the 30 subjects was computed using the Food Composition Table from Nutritive Value of Indian foods given by ICMR (Gopalan, 2007). From this the amount of nutrients namely energy, carbohydrate, protein, fat, iron, beta carotene and vitamin C consumed was calculated.

3.5.2.5 Clinical assessment

Clinical examination forms an essential part of assessment of nutritional status. It was done with the help of an oncologist. Clinical symptoms such as dull hair, sparse hair, dull skin, dry skin, tooth decay, oral cavity lesion, mouth ulcer and dysplasia were recorded.

3.5.2.6 Study the impact of the seaweed green tea among adult male subjects with precancerous oral lesions

Seaweed tea 100ml/day was given to the experimental group of precancerous oral lesions and plain black tea was given for the control group for a period of 30 days. The subjects were asked not to take extra cups of other beverages like coffee, tea other than the supplement tea. The biochemical parameters like haemoglobin, serum iron, red blood cells, white blood cells, serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase, alkaline phosphatase, catalase and super oxide dismutase were analysed to study the antioxidant effect. The procedures used are given in Appendix III and IX. In addition to the biochemical results the lesions were reviewed by the oncologist for any change in the appearance after the supplementation.
3.5.2.7 Data analysis

Statistical analysis of data was done using mean, standard deviation, t-test, F value and ANACOVA to find the level of significance on the effect of supplementation of seaweed chocolate and seaweed tea on selected anaemic adolescent girls and male adults with precancerous oral lesions. All the statistical analysis was done using Statistical Package for Social Sciences version 9.0. Adjusted mean calculation used in ANACOVA is given in Appendix XIV.