Seaweeds offer untapped plethora of health benefits. The long recognized traditional health benefits of certain seaweeds are now being confirmed by modern scientific research. Seaweeds are being used for its rich nutrient content and antioxidant property in treating major degenerative and deficiency diseases. Though seaweeds are consumed extensively by Indonesians, Japanese and Koreans who have understood the nutritional properties and valuable health benefits of seaweeds Indians are yet to explore its benefits. It is reported that seaweeds like *Ulva lactuca*, *Ulva reticulata*, *Enteromorpha intestinalis*, *Acanthophora spicefera*, *Gracilaria edulis*, *Padina tetrastomatica* and *Sargassum wightii* are highly concentrated in the coastal belt of Gulf of Mannar, Rameswaram to Kanniyakumari in Tamil Nadu.

In India nutritional deficiency and degenerative diseases are widespread. There is high incidence of oral cancer especially in rural areas. Specifically 60-70 per cent of the adolescents in India are reported to suffer from iron deficiency anaemia. Fresh, natural and minimally processed quality foods can help improve the nutritional value and health of the population. Hence the present study entitled “Nutrient Composition, Antioxidant Activity and Therapeutic Use of Selected Seaweeds” was undertaken with the objectives: To

1) assess the nutrient and microbial content, evaluate the toxicology, antioxidant activity and antimicrobial properties of the selected seaweed

2) develop value added products with seaweeds and study the *invitro* iron bioavailability and antioxidant activity of the value added seaweed products

3) study the impact of value added seaweed products among selected anaemic adolescent girls and adult males with precancerous oral lesions.
Four seaweeds namely one brown (*Padina tetrastomatica*), one red (*Acanthophora spicefera*) and two green (*Ulva lactuca* and *Ulva reticulata*) were collected from Pamban and Thonithurai the two important coastal zones of Gulf of Mannar, where seaweeds are abundant. Seaweeds were collected fresh with the help of sea divers trained in handpicking these seaweeds. The collected seaweeds were thoroughly cleaned washed and shade dried. The morphology, nutrient composition namely proximate composition, mineral and vitamin composition, heavy metal and bioactive compounds were analysed using standard procedures.

The seaweeds were subjected to microbial analysis namely total bacterial count and tested for the presence of *Escherichia coli*, *Salmonella*, *Bacillus* and *Pseudomonas*. Toxicological evaluation was carried out by conducting acute and sub acute toxicological studies using animal model. The two green seaweeds *Ulva lactuca* and *Ulva reticulata* were found to be safe even at higher levels of dosing (5000 mg/kg body weight) thus these two seaweeds were selected for further investigations.

Antioxidant and antimicrobial activity was carried out for the two green seaweeds. The antioxidant activity was determined using DPPH radical scavenging method and Butylated Hydroxyl Toluene was used as the standard. Common food pathogens namely *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Psuedomonas aeruginosa*, *Shigella dysenteriae*, *Vibrio cholera* and *Psuedomonas mirabilis* and two fungi namely *Candida mirabilis* and *Candida tropicalis* were tested. Chloramphenicol and miconazole were used as standard for antibacterial and antifungal activity respectively. The estimation of the Minimal Inhibitory Concentration (MIC) was carried out by the broth dilution method.

Value added products was developed using the two green seaweeds namely *Ulva lactuca* and *Ulva reticulata*, which were found to be safe. Seven recipes each incorporating *Ulva lactuca* and *Ulva reticulata* were developed. Tomato soup,
vegetable soup, tomato spread, seaweed tea, chocolate, nutrient ball and bun were
the recipes selected. The recipes were standardised and subjected to consumer
acceptability by one hundred students belonging to a private college selected
for the study. Scores were allotted for the attributes like appearance, colour,
texture/consistency, flavour and taste. The product with the highest score for overall
acceptability was selected for supplementation. Five gram *Ulva lactuca*
incorporated seaweed tea and five gram *Ulva reticulata* incorporated seaweed
chocolate was taken for supplementation.

Prior to administration of the value added seaweed tea and seaweed
chocolate the nutrient, phytonutrient, *invitro* iron bioavailability and *invivo*
antioxidant activity was studied.

Nutrient content namely protein, lipid, carbohydrate and iron was analysed.
The energy value was computed using the nutritive value of Indian foods. *Invitro*
iron availability was estimated separately for *Ulva reticulata* extract, plain
chocolate and *Ulva reticulata* incorporated chocolate.

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 criteria of WHO, 2001 was used
to categorize the mild, moderate and severe anaemic students. The selected girls
were divided into two with each fifty subjects as control and experimental. The
socioeconomic and nutritional status was assessed for all the selected subjects. The
experimental group received 20 g seaweed chocolate consisting of five gram *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 to be
supplemented for anaemic adolescent girls. The control group received 15gm
chocolate without the seaweed. Before supplementation all the subjects were
dewormed and biochemical analysis were carried out on the initial and final day for
the following biochemical parameters like haemoglobin, mean corpuscular volume,
mean corpuscular haemoglobin, red blood cell, white blood cell, total iron binding capacity, serum iron and serum ferritin. Haemoglobin test alone was carried out for all the subjects on the initial, 30th and 120th day.

Seaweed tea was subjected to phytonutrient analysis and to *in vivo* antioxidant activity on animal model. Rats were randomly divided into six groups of six animals each and supplemented with 0.5, 1.0, 1.5 and 2.0ml/kg body weight of seaweed tea and vitamin C was used as a standard (3.0ml/kg body weight) and saline as control. The rats were fed for a period of 20 days. After a period of 20 days the blood from the animals were drawn and tested for antioxidant enzymes namely superoxide dismutase, lipid peroxidase, catalase and glutathione peroxidase.

The seaweed tea showed a remarkable antioxidant property. Thus the product was administered for adults with precancerous lesions. In order to identify the patients a community health camp was organized in a private hospital, Kilakarai and patients were reviewed by an oncologist. Among the 650 beneficiaries 40 male subjects showed signs of dysplasia (Abnormal development or growth of tissues, organs, or cells) and from the forty, thirty subjects who were willing to participate in the study were selected.

Adults with precancerous oral lesions were males in the age group of 30-45 years and were divided into two groups 15 as experimental and 15 as control.

Seaweed tea (100ml/day was given) for the experimental group and plain black tea (100ml/day) was given for the control group for a period of 30 days. 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 studied on the initial and final day of supplementation. In addition to the biochemical results the precancerous lesions were reviewed by the oncologist for any change in the appearance.
The findings of the study are summarised below

Morphological characteristics, nutrient composition and microbial content

- The observed morphological characteristics of the selected edible seaweeds showed that, *Padina tetrastomatica* is a dark brown coloured fan shaped seaweed with several thick lines on the thallus with leathery and coarse texture always seen attached to corals.

- *Ulva lactuca*, green algae is a bright green sheet and silky to touch. The shapes of *Ulva lactuca* is quite variable but almost observed as circular or oval with waved margins and has few holes in its blade and are free floating.

- *Ulva reticulata*, green algae is a grass green flat blade of long strips with distinctive holes. It looks more like a filamentous spring. This seaweed is mostly observed as free floating.

- *Acanthophra spicefera*, red algae grows in a upright clumps of spiny branches. It is pale yellow, brownish, dark green or reddish in colour. Grows on the reef in intertidal regions.

- The proximate nutrient composition showed that *Acanthophora spicefera* had the highest amount of carbohydrate of 28 g while *Padina* had the lowest with 8.8g. The protein content of the four seaweeds ranged from 27-37.6 g/100 g with *Padina tetrastomatica* having the highest amount of protein (37.6g) followed by *Acanthophora* (34g), *Ulva lactuca* (29g) and *Ulva reticulata* (27.4g).

- Lipid content of the seaweeds was found to be in the range of 0.4 to 5 g with the least amount in *Padina* (0.4g) and the highest amount in *Acanthophora spicefera* (5g).
• The fiber content was highest in *Padina tetrastomatica* with 45g and *Acanthophora spicefera* (37g) followed by *Ulva lactuca* and *Ulva reticulata* with 31 and 29g respectively.

• Micronutrients like iron (50.3mg), calcium (81.6mg), phosphorous (12mg), sodium (32mg), zinc (1.9mg) and selenium (3.2g) were highest in *Ulva reticulata* followed by *Ulva lactuca*. Vitamin C was highest (32.4mg) in *Ulva lactuca* followed by *Ulva reticulata* (31mg).

• The betacarotene content of *Padina tetrastomatica* and *Ulva lactuca* was 9µg and 7.45µg respectively.

• The concentration of heavy metals such as mercury, lead, arsenic and cadmium was at a very low and below detectable level (BDL).

• Bioactive compounds present in the selected four seaweeds showed the presence of various fractions of fatty acid and plant alcohol like n-Hexadecaenoic acid, phytol, linoleic acid, tetradecaenoic acid, 9– Octadecaenoic acid, n-Decanoic acid and 1,2- Benzene dicarboxylic acid.

• The total bacterial count of the selected seaweeds was low in *Ulva lactuca* with 22 cfu /100gm and the highest colony count among the four selected seaweeds was observed in *Acanthophora spicefera* with 65 cfu/100gm. The qualitative analysis of the selected seaweeds revealed the absence of *Escherichia coli*, *Salmonella*, *Bacillus* and *Pseudomonas*.

**Toxicological results, antioxidant and antimicrobial activity**

• Acute and subacute toxicological evaluation was done to find the safety of seaweeds for human consumption

• Clinical signs of toxicity on administration of 2000mg /kg body weight of seaweeds in rats were observed. Among the four seaweeds the two green
seaweeds *Ulva lactuca* and *Ulva reticulata* administered animals showed normal behavior and clinical signs.

- Administration of seaweeds orally to rats at higher dose of 5000mg/kg body weight caused mild to severe depression in the rats fed with *Padina tetrastomatica* and *Acanthophora spicifera*.

- Administration of seaweeds *Ulva lactuca* and *Ulva reticulata* orally to rats at higher dose of 5000mg/kg showed a normal behavior from the time of dosing till the final day of observation. Hence green seaweeds were considered safe even at higher dosing levels of 5000mg/kgbw and therefore were used for further research.

**Sub acute toxicity**

- Physical changes were observed in the animals after 60 days of supplementation. In all the three groups the animals were found to be active, consumed food and water normally and had normal body temperature. There was no adverse changes seen in the rats.

- The total ferritin levels were comparatively high in seaweed administered rats with 119±3.60 and 131.67±2.08 as against 111.3±3.21 of control group. The serum iron increased in *Ulva reticulata* supplemented animals (121.67±2.08) than in control and *Ulva lactuca* supplemented animals.

- Minerals like sodium, potassium, chloride, phosphorus, calcium and magnesium, increased in experimental group compared to control group. The increase in mineral content in *Ulva reticulata* supplemented animals may be attributed to the high mineral content naturally present in the seaweeds

- The serum marker enzymes such as SGOT, SGPT and ALP were found to be within normal limits in both the seaweed supplemented group.
• Normal blood parameters were seen in seaweed supplemented rats and this was a positive indication that these natural foods are safe for human consumption.

**Antioxidant and antimicrobial activity**

• The results of the antioxidant activity of the seaweeds indicate a positive DPPH radical scavenging activity, for *Ulva lactuca* (72 per cent) and *Ulva reticulata* (78 per cent) against the BHT (Butylated Hydroxyl Toluene) (60 per cent).

• The investigations on ethanol extracts of *Ulva lactuca* and *Ulva reticulata* showed maximum activity against *Staphylococcus aureus* (15.0±0.50) Methanol extract of *Ulva lactuca* showed maximum activity against *Staphylococcus aureus* (16.03±0.45) and *P. aeruginosa* (15.10±0.45). The antifungal activity of ethanol extract of *Ulva lactuca* seaweed showed maximum activity against *Candida albicans* (12.07±0.40) and *Ulva reticulata* showed maximum activity against *Candida tropicalis* (10.97±0.45).

• Both the seaweeds showed minimum inhibitory concentration at 250 mg/ml concentration.

**Value added products with the incorporation of green seaweeds and acceptability of the products**

• Seaweed incorporated recipes had a comparatively higher overall acceptability than the standard recipes except for bun. *Ulva reticulata*, seaweed chocolate obtained the highest score of 24. Among the *Ulva lactuca* incorporated products seaweed green tea had a high score of 23 followed by seaweed chocolate (19). Thus *Ulva reticulata* incorporated chocolate and *Ulva lactuca* incorporated tea was selected for further study.
Nutrient content and Invitro Iron bioavailability of Seaweed chocolate

- The seaweed chocolate per 100g had carbohydrate (30g), fat (8.9g), protein (10.9g) and 243 kcal of energy. The iron content of chocolate was 56.24mg.

- The invitro iron bioavailability in seaweed chocolate was 21 per cent. Thus seaweed chocolate was selected for supplementation to anaemic adolescent girls.

Impact of seaweed chocolate on anaemic adolescent girls

- The selected adolescent girl were in the age group of 15-18 yrs the majority forty eight per cent being in the 15-16 year group.

- The pubertal age of 11 years was seen commonly among the selected girls.

- Fifteen girls were underweight and 80 per cent of the girls were normal in the fifth to 50th per centile and only five girls were in the 85th per centile indicating that they have a risk of overweight.

- The meal pattern of the selected girls showed that 66 per cent were non-vegetarians, 19 per cent were ova-vegetarian and 15 per cent were vegetarians.

- Mean nutrient intake of the selected adolescent girls indicated that their intake was deficit in energy, protein, calcium, iron, beta carotene and vitamin C when compared to the Recommended Dietary Allowance.

- Clinical signs observed were dry hair, pale eye pallor, dry skin and dryness of mouth. All the signs observed were related to iron deficiency anaemia.

- The impact of seaweed chocolate on haemoglobin levels of the selected subjects showed that haemoglobin levels increased only gradually in the experimental group after 120 days (8.53±0.27 to 9.60±0.59).
• Mean corpuscular volume and mean corpuscular hemoglobin of the selected adolescent girls in control and experimental groups was assessed and was found to have a significant difference between initial and final.

• The increase in red blood cell count ranged from 0.7 – 1.1 in the control and experimental groups respectively. In the experimental group the initial value was 4.3 and it increased to 5.4. The white blood cell count of the subjects in experimental group increased from 6.0-6.9 x10³µl.

• On supplementation of seaweed chocolate to anaemic adolescent girls there was a significant increase in total iron binding capacity (TIBC) levels of the experimental group than the control group.

• Supplementation of seaweed chocolate improved the serum iron and ferritin status and there was a significant difference (t<0.01) between initial and final values of serum iron and serum ferritin levels.

Phytonutrient and *In vivo* antioxidant activity

• *Ulva lactuca* incorporated seaweed tea had the following phytochemicals namely polyphenol (140mg), chlorophyll (0.273mg), tannins (52.5mg) and beta carotene (0.666IU).

• Among the four seaweed tea catalase, glutathione and superoxide dismutase activity was maximum for A4 (130±4.96, 11.55±0.64 and 20.15±1.06). The lipid peroxidase levels were low in the standard supplemented Vitamin C (1.16±0.07) treated group followed by A4 (1.77±0.058). As the quantity of seaweed tea fed to the rats increased, the concentration of the bioactive compounds also increased.

Impact of seaweed tea on precancerous oral lesions

• Among the selected 30 male subjects (17) were in the age group of 40-45 years.
• Twenty four of the selected adults with precancerous oral lesions were living in nuclear family system.

• The monthly income of seventeen subjects was Rs1000-4000 and for eight subjects it ranged from Rs 4000-8000. Fishing was the major occupation of the subjects.

• Data on the lifestyle habits of the selected subjects revealed that tobacco was used by all in the form of cigarettes/beedis or chewing tobacco.

• Nearly 77 per cent of the subjects in both the experimental and control groups had normal Body Mass Index.

• Clinical assessment of the selected subjects revealed mouth ulcers, dysplasia and oral cavity lesions was observed in all the selected 30 patients

• Mouth ulcer (25), oral cavity lesions (30) tooth discoloration (26), tooth decay (21) and pale pallor (26) were noted. All the selected subjects had oral lesions and dysplasia seen on the tongue or cheek.

• Except for fat intake all the other nutrient intake was deficit.

• The marker enzymes like SGPT (U/L), SGOT (U/L) and ALP (U/L), decreased well from 38.70±1.54 to 32.90±1.54, 32.90±1.54 to 30.00±1.58 and 52.94±0.71 to 47.30±0.68 respectively in the experimental group.

• The levels of antioxidant enzymes namely catalase (U/mg/dl) (4.95±0.81 to 5.95±0.81) and SOD (U/mg/dl) (1.22±0.12 to 1.55±0.12) increased in experimental group. In the present study antioxidant enzyme levels have appreciably increased and indicate the potential of green algae in treatment of oral cancer patients.
CONCLUSION

Seaweeds have been consumed by people dating back to 100 centuries but they have never found their rightful place in the daily diets of our population. The consumption of seaweed is sporadically seen near the coastal areas but is still underexploited. Seaweed products are used commercially but as a natural food the goodness of seaweeds have to be utilized.

The research points out that seaweed contains substantial amount of protein, fat, carbohydrate and essential minerals iron, calcium, selenium, phosphorus, zinc, sodium and magnesium and essential vitamins C and A. The heavy metal composition below the detectable level indicates the safety of these seaweeds. The toxicological evaluation of the seaweed brings forth the fact that they are safe for human consumption at five gram levels and can be consumed upto ten gram everyday.

The antioxidant and antimicrobial studies clearly point out that seaweed can be used to enhance immunity and prevent oxidative stress among people.

The therapeutic effect of seaweeds shows a promising result. Seaweeds could be an ideal food adjunct and be used to combat iron deficiency at large. So also the curative effect on precancerous lesions is significantly proved and hence can go a long way to be used as a potential functional food. As nature’s wealth seaweeds have to be used to promote future health.

Scope for future work

- Amino acids and nucleic acids present in the seaweeds can be analysed
- Studies using seaweeds as a preservative
- Encapsulation studies can be carried out to make products out of seaweeds more palatable.