SUMMARY AND CONCLUSION

In Asia, seaweeds have been consumed as a vegetable since the beginning of time. This ancient tradition of consuming seaweeds have shown various health benefits. In addition, seaweeds constitute an interesting source of nutraceutical potentials with health protective effects. Hence the present study entitled “Nutritional and Safety Evaluation of Underexploited Seaweeds and Nutraceutical Potentials of Ulva fasciata” was conducted with the specific objectives of determining the nutrient and nutraceutical potentials of the selected seaweeds and also to evaluate the sub – chronic toxicity of A.spicifera, G.edulis, P.gymnospora, Ulva fasciata and E.flexuosa in albino rats and study the hypoglycemic potentials and anticancer potential of Ulva fasciata in mice. These seaweeds were collected from sea divers and cultivation sites of the coastal area. These collected of seaweeds were thoroughly washed in seawater and tap water, then these were dried and powdered. From this, methanolic and aqueous extracts were prepared.

In Phase I, seaweeds were analysed for macro nutrients and micro nutrients like carbohydrates, protein, fat, ash, calcium, iron, phosphrous, magnesium, iodine, riboflavin and niacin using standard procedures. Antioxidants and phytochemicals were also analysed. Antioxidants such as chlorophyll a, chlorophyll b, total chlorophyll, total carotenoid, beta carotene and vitamin C were analysed by using standard procedures. In vitro antioxidant assays in terms of total phenol content, DPPH and nitric oxide were analyzed for the underexploited seaweeds. The phytochemical screening of the selected seaweeds were qualitatively analysed by standard techniques. Microbial load for the presence of E.coli, S.aureus, Streptococci, Salmonella typhii, and standard plate count and coliforms were tested using standard procedures to confirm the safety of seaweeds. Antibacterial and
antifungal activity was also carried out for the methanolic extract of underexploited seaweeds by standard techniques.

In order to assess the safety of consumption of seaweeds, in phase II, sub – chronic toxicity of underexploited seaweeds was tested in albino rats as per OECD guidelines for a period of 90 days. A group of healthy albino rats of wistar strain were obtained from the animal laboratory. After acclimatization, rats were assigned to six groups of six each As per OECD guidelines 40mg of seaweed extracts were given orally to the rats in experimental groups. The body weight gain and food consumption was recorded on a weekly basis and clinical signs like mortality and morbidity of rats were observed daily. On the 90th day, rats were sacrificed and the blood samples were collected by retro orbital plexus technique from all the groups, which were analyzed for various hematological parameters (Hemoglobin, PCV, RBC, WBC, PCV, MCV, MCH and MCHC) and biochemical parameters (Glucose, cholesterol, blood urea nitrogen, albumin, SGOT, SGPT, sodium, potassium, calcium, phosphorus and alkaline phosphatase). Urine electrolytes (pH, sodium, potassium and chloride) and histopathological examinations were performed. Heavy metals like cadmium, mercury, arsenic and lead were analyzed in organs like brain, kidney and liver of the rats.

In Phase III, acceptability trial was carried out for the selected seaweeds by incorporating seaweeds in traditional recipes like vegetarian and non vegetarian dishes namely seaweed soup, rasam, chappathi, dosa, poriyal, pickle, chicken biriyani, scrambled egg, fish curry, and chicken gravy. A five point hedonic scale was developed to evaluate the appearance, flavour, texture and overall acceptability. Results revealed Ulva fasciata to be more acceptable than other seaweeds. Therefore, in phase IV, antidiabetic activity was evaluated for Ulva fasciata. A total of 30 rats (24 diabetic surviving rats and six normal rats) were used. Alloxan monohydrate was given to induce diabetes in rats. The rats were divided into five groups after the induction of
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diabetes. Aqueous extract of *Ulva fasciata* was given in two different doses 200mg/kg and 400mg/kg to experimental groups (group IV and V) for a period of 28 days. Body weight and blood glucose was noted weekly. Liver glycogen, glycosylated hemoglobin, carbohydrate metabolic enzymes like hexokinase, glucokinase and glucose 6 phosphatase were assessed.

In phase V, nanoparticle was developed from *Ulva fasciata* and it was characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM) and FTIR. In Phase VI, the developed nanoparticles and crude extract of *Ulva fasciata* (methanol and aqueous extract) were studied for cancer activity against Dalton’s Ascites Lymphoma (DAL) cell lines in vivo for a period of 14 days. Cell count, per cent increase in life span (%ILS), increase in body weight were assessed. Hematological parameters (WBC, RBC, PCV and platelets) and biochemical parameters (cholesterol, TGL, AST, ALT and ALP) were evaluated.

The data was subjected to statistical analysis using Graphpad Prism. One Way ANOVA was used to interpret intra group variations for different parameters. Dunnett’s multiple comparison test was applied to interpret the biochemical and hematological parameters of all groups in toxicological study. Newman Keul’s multiple range tests was used to determine One way ANOVA to compare mean of the haematological and biochemical parameters of the control and experimental groups to determine the significant difference between the groups.

The salient findings of the study are summarized below:

- The carbohydrate content of the selected seaweeds varied from 24.8g to 32g. The maximum carbohydrate content was recorded in *Ulva fasciata* (32g) followed by *Enteromorpha flexuosa* (30.10g), *Padina gymnospora* (28g) *Acanthophora spicifera* (26.20g) and the minimum was found in *Gracilara edulis* (24.80g).
Protein content varied from 12.07 to 22.78 per cent, maximum protein was recorded in *U.fasciata* (22.78g), *A.spicifera* (20.2g), *G.edulis* (18.04g), *E.flexuosa* (12.29g) and minimum was observed in *P.gymnospora* (12.07g).

The total lipid contents in the samples ranged from 0.48-1.4g. The lipid content was high in *P.gymnospora* (1.4g) followed by *U.fasciata* (0.89g) and least amount was found in *A.spicifera* (0.48g).

Ash content of the selected seaweeds was in the level of 21 to 32.20g which was considered to be high. High level of ash was associated with the amount of mineral elements. Ash content in *Enteromorpha flexuosa* (32.20g) was maximum followed by *Ulva fasciata* (27g). The least amount was found in red seaweed namely *Acanthophora spicifera* (21g).

The calcium content of the selected seaweeds was in the range 410 to 820mg, the maximum concentration was found to be in *P.gymnospora* (820mg) and minimum concentration in *G.edulis* (410mg). In case of phosphorus, it was high in *E.flexuosa* (270mg) followed by *A.spicifera* 210mg, *P.gymnospora* (164mg) and least values were recorded in *U.fasciata* (142mg) and *G.edulis* (124mg).

Iron content was in the range 14.8 to 72mg, it was found to be high in *G.edulis*, followed by *A.spicifera* (52mg), *U.fasciata* (47mg), *E.flexuosa* (40mg) and low level in *P.gymnospora* (14.8mg).

Magnesium was in the range of 420 to 780mg, which was high in *P.gymnospora* (780mg), *G.edulis* (580mg), *A.spicifera* (480.02mg) and least in green seaweeds namely *U.fasciata* (420mg) *E.flexuosa* (436mg).

In the present study, sodium and potassium accumulation was found to be high in the selected seaweeds. In case of red seaweeds, *G.edulis* potassium was 52.12mg and sodium was 32.03mg and *A.spicifera*...
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contained 52mg of potassium and 36mg of sodium respectively. Brown seaweed *P.gymnospora* contained 36.36mg of sodium and 30.0mg of potassium. On the other hand, green seaweeds contained low sodium and potassium when compared to the other seaweeds *U.fasciata* contained 20.12mg of sodium and 27.20mg of potassium *E.flexuosa* contained 13.20mg of sodium and 22.32mg of potassium respectively.

The selected seaweeds contained 38.89 to 72.20mg/100g of iodine respectively, which was high in *G.edulis* (72.20mg) followed by *A.spicifera* (64.8mg), *P.gymnospora* (46.2mg) and low in *E.flexuosa* (42.3mg) and *Ulva fasciata* (38.89mg).

Niacin was found to be higher in green seaweeds than red and brown seaweeds. *U.fasciata* and *E.flexuosa* contained 1.02mg and 0.92mg of niacin respectively, whereas red seaweed *A.spicifera* contained 0.78mg and *G.edulis* 0.52mg of niacin. On the other hand, brown seaweed *P.gymnospora* contained very low level (0.34mg) of niacin.

Riboflavin was in the range of 0.08mg to 0.32mg, which was recorded highest in *U.fasciata* (0.32mg) followed by *E.flexuosa* (0.26mg) *A.spicifera* (0.14mg), *G.edulis* (0.12mg) and the least found in *P.gymnospora* (0.12mg).

The mercury level was not detected in any of the selected seaweeds except *A.spicifera*, in which it was 0.031ppm. Arsenic content was in the range of 0.026ppm to 0.212ppm, which was highest in *A.spicifera* and lowest in *U.fasciata*. The cadmium content was in the range of 0.012ppm to 0.081ppm, and was found to be highest in the *P.gymnospora* and lowest in the *G.edulis*. Lead was not detected in *A.spicifera*, *U.fasciata* and *E.flexuosa*. On the other hand, the lead content in *G.edulis* was 0.196ppm and that of *P.gymnospora* was 0.103ppm.
Copper concentration in the samples ranged from 0.025ppm to 0.979ppm and maximum was seen in G.edulis 0.979ppm and lowest concentration found in E.flexuosa (0.025ppm).

Concentration of zinc in the selected seaweeds were observed to be maximum in E.flexuosa (1.518ppm) followed by U.fasciata (1.342ppm), G.edulis (1.210ppm), P.gymnospora (1.187ppm) and minimum amount in A.spicifera (1.080ppm).

The chlorophyll content was highest in green seaweeds followed by brown seaweeds and the least in red seaweed which was in the range of 2.09 to 0.66mg/g fresh weight. Chlorophyll a content was found to be maximum in Ulva fasciata (2.09mg/g fresh weight) and minimum in Gracilaria edulis (0.66mg/g fresh weight)

Chlorophyll b was observed to be maximum in U.fasciata (1.40mg/g) and least in G.edulis (0.13mg/g). Amongst all the species of the three algal groups, the order of decreasing chlorophyll a content was as follows chlorophyta > phaeophyta > rhodophyta.

Total carotenoid was in the range of 0.13 to78mg/g, which was maximum in P.gymnospora and minimum in the G.edulis. Vitamin C level was found to be maximum in green seaweed U.fasciata and E.flexuosa (0.38 to 0.36mg) followed by brown seaweed P.gymnospora 0.29mg and small amount in red seaweed G.edulis and A.spicifera (0.17 to 0.25mg). Aspartic acid and glutamic acid were found to be high in all the selected seaweeds. The ratios of EAA to non-EAA of seaweeds ranged from 0.72–1.02 and their ratios of EAA to total amino acids were almost 0.41–0.5. Leucine, isoleucine and lysine were found to be high amount of EAA in all the selected seaweeds. The non-EAAs, which are histidine, aspartic acid, glutamic acid, serine, proline, glycine, and alanine were present in relatively high levels. The contents of total
amino acids ranged from 444.5–647.5mg/g protein which was found to be high in *U. fasciata* and low in *E. flexuosa* respectively.

*The selected seaweeds contained all types of fatty acids viz., saturated, monounsaturated and polyunsaturated fatty acids. G.edulis contained 0.80 per cent and *U. fasciata* contained 0.59 per cent of arachidonic acid in six carbon chain, all other seaweeds did not contain any polyunsaturated fatty acids. The simple fatty acids like capric, lauric, myristic, penta decyclic, palmitic, margaric acid and stearic acid were present in all the selected seaweeds. The green seaweed *U. fasciata* contained high amount of monounsaturated and polyunsaturated fatty acids compared to saturated fatty acids. The brown seaweed *P. gymnospora* contain high amount of saturated fatty acids, when compared to other fatty acids and this seaweed contained more unidentified fatty acids.*

*The phytochemical screening of all the selected seaweeds showed that the seaweeds contained carbohydrates, protein, flavonoids, sterols, saponins, tannins, gums, mucilage, terpenoids, phenols, and starch. Alkaloids were absent in all the selected seaweeds. Glycosides was absent in *P. gymnospora*. Quinones was absent in the brown seaweed namely *P. gymnospora* and green seaweed such as *U. fasciata*, and *E. flexuosa*. The microbial analysis revealed that there was no detectable *Salmonella typhii* and faecal streptococci in any of the selected seaweeds. Coliforms was in the range of <1/g to <2/g, which was not detected in *P. gymnospora*. Staphylococcus was detected in red seaweed namely *A. spicifera*, brown seaweed *P. gymnospora* and green seaweed *E. flexuosa*, whereas *E. coli* was <3 /g in *E. flexuosa* and <1/g in *Padina gymnospora*. The standard plate count in red seaweeds was found to be 30 cfu/g for *Acanthophora spicifera* and 90 cfu/g for...*
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*Gracilaria edulis*, brown seaweed *P. gymnospora* it was 20 cfu/g and for green seaweeds *U. fasciata* it was 25 cfu/g and *E. flexuosa* it was 43cfu/g. The counts were within the safe limits prescribed thereby indicating the safety in consumption of seaweeds.

For antibacterial activity, the largest zone was found in methanolic extract of *Acanthophora spicifera* and *Ulva fasciata* against *S. aureus* at 60µg/disc concentration. The maximum zone of inhibition was against *Streptococci* for the methanolic extract of *E. flexuosa*. *Ulva fasciata* showed strong activity against *E. coli* (19mm) at 60µg/disc concentration. All other seaweed extracts showed moderate activity against the tested organisms. *Ulva fasciata* and *Enteromorpha flexuosa* showed strong activity against *Proteus vulgaris* at similar concentration. The results proved the antibacterial activity of the selected seaweeds.

The red seaweed *G. edulis* and green seaweed *E. flexuosa* showed strong activity against *Klebsiella aerogenes* at 60µg/disc concentration.

The antifungal activity showed that the methanol extracts of all the selected seaweeds showed good activity against *C. albicans* and *A. niger*. The highest zones of inhibition was found in *A. spicifera* and *U. fasciata* (16mm), whereas all other seaweeds showed moderate and weak activity against *Aspergillus niger*. For activity against *Candida albicans*, only moderate activity was noticed in *A. spicifera*, *G. edulis*, *P. gymnospora* and *E. flexuosa*.

*In vitro* antioxidant activity revealed that the total phenol content in methanol extract of *Ulva fasciata* showed the maximum amount (23mgTAE/g) followed by *Gracilaria edulis* (15.0mgTAE/g), *Padina gymnospora* (12mgTAE/g), *Enteromorpha flexuosa* (11.4mgTAE/g), and least in *Acanthophora spicifera* (8.8mgTAE/g).
The DPPH radical scavenging activity showed that the inhibition of all the selected seaweed extracts decreased in the following order *Ulva fasciata* > *Gracilaria edulis* > *Padina gymnospora* > *Enteromorpha flexuosa* > *Acanthophora spicifera*. The IC\textsubscript{50} values of methanolic extract of *Ulva fasciata* showed excellent activity (11\(\mu\)g/ml) followed by *Gracilaria edulis* (16\(\mu\)g/ml). The extracts of *P.gymnospora* showed moderate activity (20\(\mu\)g/ml) and minimum activity was found in *Enteromorpha flexuosa* and *Acanthophora spicifera* which was 42\(\mu\)g/ml and 47\(\mu\)g/ml respectively. The IC\textsubscript{50} value for BHA was 4.7\(\mu\)g/ml.

The results of nitric oxide radical activity showed that *G.edulis* (100\(\mu\)g/ml) had scavenging activity (36.3\%) followed by *A.spicifera* (29.3\%), *P.gymnospora* (23.6), *U.fasciata* (17.2\%) and *E.flexuosa* (12.2\%). These results proved the antioxidant activity of the selected seaweeds.

Regarding the sub-chronic toxicity in albino rats, results revealed that the initial body weight of the albino rats ranged from 235.32g to 240.47g and the mean weights were similar among all the groups of albino rats. At the end of the study period, it was noticed that the weight gain was higher (54\%) in experimental group IV supplemented with *Ulva fasciata* when compared to the control group. The other experimental groups namely I, II, III, V showed an increase by 39 to 46.09 per cent. The mean weight gain in the control group was 348.12g (43\%) and in experimental group I, it was 328.89g, in experimental group II it was 331g, experimental group III- 342.01g, experimental group IV it was 394.27g and experimental group V 356.32g.

The increase in weight gain of seaweed extract treated group also coincides with the increased food intake in the experimental groups when compared to that of the control group. There was a significant
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difference in PCV and MCV values when compared to the control group but these differences were found to be within the normal range. No significant difference in blood parameters was observed between experimental groups treated with aqueous extract of *Acanthophora spicifera*, *Gracilaria edulis*, *Padina gymnospora*, *Ulva fasciata* and *Enteromorpha flexuosa* and control group. In general, all the biochemical values remained within the normal limits and did not show toxic effects on chronic treatment.

When compared to the control group, all the experimental group rats showed significant difference in cholesterol level but these values were within the normal levels. There was a significant increase in the potassium content of the experimental group III rats supplemented with *Padina gymnospora* and a decrease level was observed in experimental group V when compared to the control group. Though there was no adverse effect on the markers of liver toxicity (plasma levels of liver enzymes- SGOT, SGPT and ALP), there was a significant difference in SGOT level of experimental group I when compared to the control group rats but these levels were found to be in the normal range.

During the 90 days treatment period with the selected underexploited seaweeds, no mortality was observed among the rats. Initially, the experimental group showed hyperactivity and sneezing and no adverse clinical manifestations like diarrhoea, hematuria and dysuria were observed in the all the five groups of treated rats during the dosage period. There were no changes in the nature of stool, urine and eye ball colour of all the albino rats.

There were no significant change in the weight of the internal vital organs of the experimental groups as compared to the control. The histopathological observation of all treated rats for experimental and
control groups showed no obvious abnormalities and the results were similar to the control group. The selected seaweeds did not reveal any toxicological lesions. The normal histological section of liver showed the normal well arranged cells and clear central vein. The normal kidney section shows the well arranged cells and glomerular basement membrane to be compact. The spleen examination showed splenic tissue that consisted of sinuses and cords. The normal heart tissue showed that the cardiac muscle cells lie in the middle of the cells.

- The urine pH ranged from 7.54 - 8.1 and *Ulva fasciata* supplemented rat had a higher pH of 8.1. Calcium level of the experimental group III was found to be high when compared to the control group and this increment may be due to the high level of calcium content in the seaweed *P.gymnospora*.

- The overall acceptability of all recipes prepared by incorporating 5-15 per cent of seaweeds, revealed that five per cent was most acceptable when compared to the 10 – 15 per cent incorporation. *U.fasciata* incorporated soup, rasam, poriyal and pickle obtained maximum scores and found to be highly acceptable followed by *E.flexuosa* and *G.edulis*.

- Among the prepared recipes the overall acceptability of non vegetarian recipes were found to be extremely good. This showed that the seaweed blends very well with non vegetarian items.

- The antidiabetic activity of *Ulva fasciata* revealed that the initial body weight of the rats were in the range of 195.83 to 206.66g before supplementation of *Ulva fasciata*. The final mean body weight of diabetic control group rats was 157.33g which showed a significant decrease when compared to normal group. The body weight of diabetic rats treated with aqueous extract of *U.fasciata* at different doses (group IV and group V) significantly increased from 206.66g to 212.33g in group IV and from 196.6g to 221.0g in group V respectively. Group III
rats treated with glipizide also showed a significant increase (227.33g) in body weight as compared to the diabetic control group rats.

The glycogen content of group II diabetic control rats was 8.05mg/g and this level was found to be very low when compared to the normal control. Improvement in liver glycogen was noticed in group IV and V rats after chronic treatment with aqueous extract of Ulva fasciata. These changes were statistically significant when compared to the normal control (Group I) and diabetic control (group II) and there was no significant change between these two groups.

The glycosylated hemoglobin of diabetic rats treated with Ulva fasciata at lower and higher doses decreased to 6.76 per cent and 5.91 per cent in group IV and group V rats respectively. In group II, the level was 8.58 per cent, when compared to the normal group (5.38%). But changes were not statistically significant, whereas the (group III) glipizide treated rats showed low level of glycosylated hemoglobin (5.73%) which was nearer to the normal levels.

In diabetic rats, blood glucose level was reduced by 40.21 per cent and 48.07 per cent in group IV and group V respectively. The standard oral hypoglycemic drug glipizide showed potent antidiabetic activity by reducing blood glucose level by 56.97 per cent. The aqueous extract of Ulva fasciata administered in two doses indicated that high dose of 400mg/kg dose reduced the glucose level to normal values whereas low dose 200mg/kg also showed a reduction nearing to the normal value.

In general, the mean levels of enzymes hexokinase, glucokinase and glucose-6-phosphatase decreased in diabetic control when compared to normal control. The respective percentage decrease was 56.9 per cent, 79.9 per cent and 67.2 per cent in diabetic control when compared to the normal control group. Treatment with Ulva fasciata at
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lower doses for 28 days led to a rise in the percentage of these parameters by 52.2 per cent, 5.74 per cent and 40.85 per cent and for higher dose, it was 54.44 per cent, 20.97 per cent and 46.34 per cent respectively (p<0.05) when compared to diabetic control. Also treatment with glipizide led to rise in the percentage of these parameters by 98.8 per cent, 52.34 per cent and 79.26 per cent respectively (p<0.05) as compared to diabetic control.

The histopathological examination of pancreas showed that, in group II rats (diabetic control) the cells were irregular, not well defined and defect in cell membrane. Necrosis of the cells was very clear. The numbers of islets cells were severe decreased, islets cells were severely swelled. In group III, pancreas the numbers of islets cells were minor decreased, islets cells were minor swelled. In case of group IV and V, the islets cells were moderated decrease with minor swelling.

In the DAL tumor control group, the average life span of animal was found to be 46 per cent MEUF and AEUF increased the life span to 76 per cent and 72 per cent respectively. However, the average life span of 5-FU treatment was found to be 92 per cent.

The cell count of the methanolic and aqueous treated groups showed significant reduction in the cancer live cells and increase the dead cell count. The total number of RBC showed modest changes in treated groups. At the same time, MEUF and AEUF treated groups restored all the altered hematological parameters to almost near normal. This may be attributed to the iron content of the seaweeds and also increased bioavailability of iron in cancer treated mice.

The inoculation of DAL cells caused significant increase in the level of total cholesterol, aspartate transaminase, alanine transaminase, alkaline phosphatase in the cancer control animals (group II), when compared to the normal group. The treatment with MEUF, AEUF and
seaweed nanoparticle reversed these changes towards the normal level. All the values were found to be significant when compared to the cancer control.

From the salient findings summarised, it may be concluded that the five underexploited seaweeds selected for the study were found to possess high nutritional value and nutraceutical potentials. Since seaweeds originate from marine source, they tend to accumulate heavy metals which may cause toxicity. Hence, conduct of sub-chronic toxicity study revealed that the underexploited seaweeds showed normal hematology and biochemistry and did not show any abnormalities in terms of histopathology. Heavy metal toxicity studies showed that the heavy metals in seaweeds did not accumulate in the organs of the rats supplemented with seaweed extracts. The heavy metal contents were also found to be within the tolerant value prescribed as safe levels. Thus the underexploited seaweeds studied were found to be safe for human consumption. Further, Ulva fasciata, the most acceptable seaweed showed a promising effect for nutraceutical potentials in terms of antidiabetic and anticancer activity.

It is recommended that future research along the following lines be undertaken:

1. Incorporation of seaweeds in weaning and supplementary foods and its protein quality assessment in animal models and children
2. Isolation of bioactive constituents and phytochemicals from different seaweeds
3. \textit{In vitro} and \textit{in vivo} studies on bioavailability of minerals and phytoconstituents in humans.
4. Indepth studies on antidiabetic potential of seaweeds in Type II diabetics
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5. Studies on mechanism of action of seaweeds on their hypoglycemic and anticancer activity

6. Popularization of seaweeds and cultivation of seaweeds to meet the food security challenges.

7. Mass media education on methods of consumption of seaweeds and storage methods.