10. SUMMARY AND CONCLUSION

Bio-prospecting or bio-diversity prospecting is defined as the “process of collecting or surveying a large set of flora (or fauna) for purpose of biological evaluation and isolation of lead compounds”. Based on the above statement, this piece of research work is mainly focused on screening the most active seaweeds available in Puducherry coast and to evaluate its bioactive potential.

Seaweeds namely, Enteromorpha compressa, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, C. antennis, Padina gymnospora, Gracilaria lithophila, and Hypnea valentiae were collected from the intertidal regions of Puducherry coast. These seaweeds were then evaluated for the presence of important phytochemicals such as alkaloids, sugars, proteins, amino acids, sterols, saponins, coumarins, flavonoids, phenols, tannins, carboxylic acids, quinones, and xanthoproteins.

Sugars, flavanoids, proteins and amino acids were present in all the eight seaweeds screened. Coumarins are present in all seaweeds analysed except G. lithophila. Saponins are present only in P. gymnospora and H. valentiae. P. gymnospora exhibited the presence of twelve phytochemicals. U. lactuca also showed the maximum phytochemicals except saponins and carboxylic acids.

Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria. Flavonoids are known as nature’s tender drug which possesses numerous biological and pharmacological activities. Steroids may serve as an intermediate for the biosynthesis of secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be a good source of un saponifiable, non toxic sterols that have medicinal value. Saponins
possess numerous biological properties which include anti-microbial, anti-inflammatory, anti-feedent and hemolytic effects. *P. gymnospora* with the presence of twelve phytochemicals analyzed, revealed the presence of various secondary metabolites with varied degree.

Upon estimation, photosynthetic pigments showed that the total chlorophyll ranged from $0.40 \pm 0.01$ to $0.75 \pm 0.01$ mgg$^{-1}$ with minimum in red seaweed, *H. valentiae* and maximum in green seaweed, *U. fasciata*. The carotenoid content ranged from $0.37 \pm 0.01$ to $0.85 \pm 0.01$ mgg$^{-1}$ with minimum in green seaweeds, *E. compressa*, *U. lactuca*, *C. antennina* and maximum in brown seaweed, *P. gymnospora*.

Biochemical composition analysis showed that, the maximum protein content was found in *P. gymnospora* (26.3%) and minimum in *C. antennina* (10.6%). Maximum lipid content was found in *U. lactuca* (2.9%) and minimum in *Padina gymnospora* (1.5%). Maximum carbohydrate content was found in *P. gymnospora* (50%) and minimum in *E. intestinalis* (18.6%).

Seaweed extracts were examined for antibacterial activity against five bacterial pathogens (*E. coli*, *S. aureus*, *P. aeruginosa*, *V. parahaemolyticus* and *K. pneumoniae*). Brown seaweed, *P. gymnospora* showed maximum zone of inhibition against all the pathogens with maximum activity against *K. pneumoniae* ($14.7 \pm 0.5$ mm) and minimum activity against *E. coli* ($10.7 \pm 0.6$ mm).

Antioxidant activities were tested using five different assays, total phenolic content, total antioxidant activity, reducing power, Hydrogen peroxide radical scavenging assay and DPPH radical scavenging activity. The total phenolic content ranged from $0.28 \pm 0.03$ to $0.89 \pm 0.02$ mg gallic acid equivalent/g with minimum in the green
seaweed *C. antennina* and maximum in the brown seaweed, *P. gymnospora*. Higher total antioxidant activity of $1.92 \pm 0.05$ mg ascorbic acid equivalent/g of seaweed was observed in *P. gymnospora*, and minimum activity of $0.64 \pm 0.03$ mg ascorbic acid equivalent/g in *C. antennina*. The maximum percentage of radical scavenging activity was observed in brown seaweed, *P. gymnospora* (90%) and minimum in *C. antennina* (25%). In the present study, reducing power increased with increasing concentration, maximum reducing power value was observed in 1 ml concentration of *P. gymnospora* ($2.678 \pm 0.03$). Hydrogen peroxide radical scavenging activity of *P. gymnospora* was recorded in highest percentage (91%) and lowest in *C. antennina* (28%).

*P. gymnospora*, brown algae showed promising results in phytochemical, pigment content, biochemical analysis and also with higher antibacterial and antioxidant activity among the seaweeds analyzed. Thus, in order to identify the bioactive compound present in *P. gymnospora*, it was extracted using acetone as the study is targeted towards bioactive pigment and fractionated through basic solvent pigment extraction, silica column chromatography and thin layer chromatography. To keep a track on the bioactivity, the fractions obtained are further analyzed for antioxidant activity.

Acetone extract was purified using silica column chromatography with solvent system, n-hexane and acetone (7:3) that yielded 12 fractions. By thin layer chromatography these fractions were grouped into major six fractions PG1, PG2, PG3, PG4, PG5, and PG6 which were then analyzed for its antioxidant activity. Based on the DPPH scavenging assay and total antioxidant activity, PG3 was considered as the most
active fraction among the six fractions. The active fraction PG3 was further purified and characterized for further study on its anti-cancer activity.

The active fraction PG3 obtained from *P. gymnospora* is partially characterized by UV-visible spectroscopy, Fourier transform infra red spectroscopy, High performance liquid chromatography and Nuclear magnetic resonance to identify the bioactive pigment present which exhibits major antioxidant activity and also for further analysis on the compound.

The UV-visible spectrum of PG3 exhibited characteristic absorption pattern ($\lambda_{\text{max}}$) in region 331 and 446 nm. The characteristic spectrum of the purified fraction PG3 was recorded by FT-IR spectroscopy. The functional groups identified in purified sample are as follows: OH group (3480 cm$^{-1}$), C–H stretch (2995–2912 cm$^{-1}$), allene (2191 cm$^{-1}$), C=O acetate (1727 cm$^{-1}$), conjugated C=O (1642 cm$^{-1}$), CH$_2$ stretch (1585–1503 cm$^{-1}$), C–C stretch in ring (1437–1407 cm$^{-1}$), germinal methyl (1307 cm$^{-1}$), C–O acetate (1257 cm$^{-1}$), and trans-distributed –C=– (1187–951 cm$^{-1}$). The HPLC chromatogram of the fraction PG3 shows a single good peak of retention time at 3.05 min, with a percentage peak area of 100% which shows the purity of the compound. The $^1$H-NMR spectra of PG3 showed 43 resonances of chemical shift (ppm).

Based on the results obtained in UV Vis spectroscopy, FT-IR, HPLC and $^1$H-NMR, the active fraction PG3 showed much similarity to the carotenoid pigment, fucoxanthin. Thus the compound present in active fraction is considered as fucoxanthin, as the results are similar to most extent. Studies on brown seaweeds rich in fucoxanthin have revealed their anti-cancer effects. Hence, the effect of fucoxanthin on cancer cells is of interest and has been studied further.
The bioactive pigment fraction PG3 of *P. gymnospora* was suspected to be fucoxanthin and is treated against cancer cell lines (MCF7 and A549) and also normal cell line (VERO) to study the anti-cancer properties of PG3 through cell line treatment for morphological changes, cytotoxicity activity by MTT assay for IC₅₀ value and DNA fragmentation for DNA damage/apoptosis.

MCF7, A549 and VERO cell lines were treated with bioactive pigment fraction, PG3, and visualized for morphological changes. The control plate did not show any morphological changes. The treated cells showed cell damage, reduction in cell volume and apoptotic bodies which were dose dependent. VERO cells showed minimal or no damage in lower concentrations. This shows that the active fraction PG3 is also selective towards carcinogenic cells. Analysis of DNA fragmentation of MCF7 and A549 cells showed that the suspected bioactive pigment fucoxanthin fragmented DNA as a feature of apoptosis. These anti-cancer properties may be related to fucoxanthin content.

Studies on brown seaweeds rich in fucoxanthin have revealed their anti-cancer effects. Hence, the effect of fucoxanthin on cancer is of interest and has been studied by several researchers. The unanimous result of the studies on fucoxanthin in cancer has established that, fucoxanthin performs a protective role and exhibits anti-proliferative behavior in various types of cancer. The biological activity of epoxycarotenoids including fucoxanthin in cancer cells grown *in vitro* and the various cellular targets of fucoxanthin has to be studied further. With the establishment of the anti-carcinogenic property of fucoxanthin, it was important to understand the mechanism by which it exerted its effect in cells. With this goal in mind, further researches to elucidate the molecules and pathways that can be modulated and regulated by fucoxanthin have to be evaluated.

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Mechanistic studies by various researchers have shown that fucoxanthin can affect many cellular processes, and may be in near future single primary mechanism of action of fucoxanthin could be established.