6. EVALUATION OF THE ANTIOXIDANT PROPERTY OF THE RED ALGAL EXTRACT (GAE) AND THE ISOLATED COMPOUNDS (GAC 1- 4) UNDER *IN VITRO* CONDITION.

6.1 Introduction

It is well established that marine natural products are rich sources of antioxidants. The aim of this chapter of the thesis was to investigate the free radical scavenging efficacy of the crude extracts and the isolated compounds from *G.acerosa.* Antioxidants play an essential role in maintaining the normal functioning of the body. As generation of free radicals is related to various diseases including cancer, this chapter analyzed the free radical scavenging activity and efficacy of *G.acerosa* to enhance the activity of SOD and POX under in vitro conditions.

6.2 Methods

As mentioned in chapter 3 of the thesis, the efficacy of *G.acerosa* to remove free radicals generated was evaluated by DPPH assay. Further the study also evaluated the efficacy of *G.acerosa* to enhance the activity of antioxidant enzymes.

6.3 Results

6.3.1 Antioxidant activity of crude algal extracts

The efficiency of the algal extracts from *G.acerosa* to scavenge free radicals was determined by DPPH assay. The results are shown in Figure 6.1.

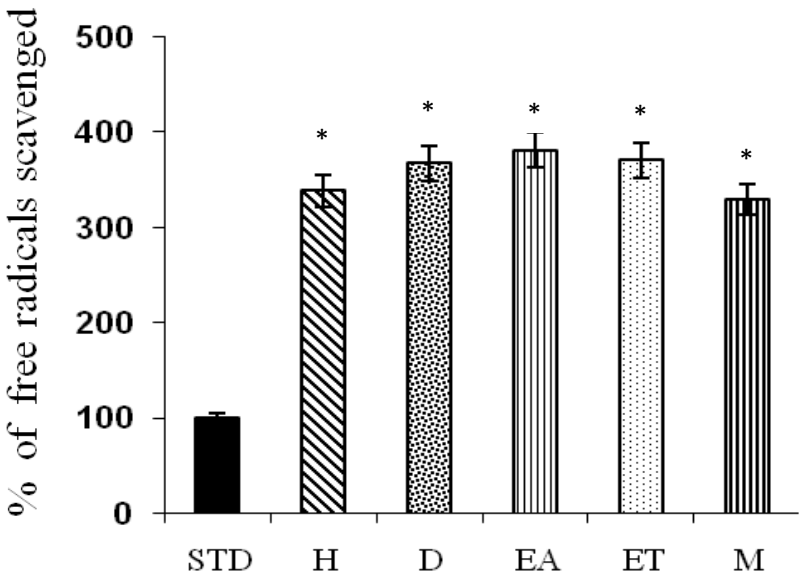

Figure 6.1 Bar charts showing the antioxidant activity of algal extracts hexane (H), DCM (D), ethyl acetate (EA), ethanol (ET) and methanol (M) extracts from *G.acerosa*.

Data are expressed as mean ± S.D, n=3, *p<0.05. The data showed that the algal extracts were more efficient in removing the free radicals when compared to standard ascorbic acid.

The outcomes of the study showed that all the algal extracts possessed free radical scavenging activity but the efficacy to remove the free radicals varied among the extracts. The highest DPPH scavenging activity was exhibited by the ethyl acetate extract (381%), followed by hexane (200%), dichloromethane (159%), ethanol (137%) and methanol (123%) extracts. Further, the results showed that the algal extracts were more efficient in scavenging the free radicals than the standard ascorbic acid.

6.3.2 Antioxidant activity of GACs

The free radical scavenging efficacy of GACs was analyzed by DPPH assay. The results are shown in Figure 6.2. Among the GACs, GAC 4 exhibited higher antioxidant activity (24%) than the other GACs. The GACs demonstrated more antioxidant efficacy than ascorbic acid and crude extract GAE.

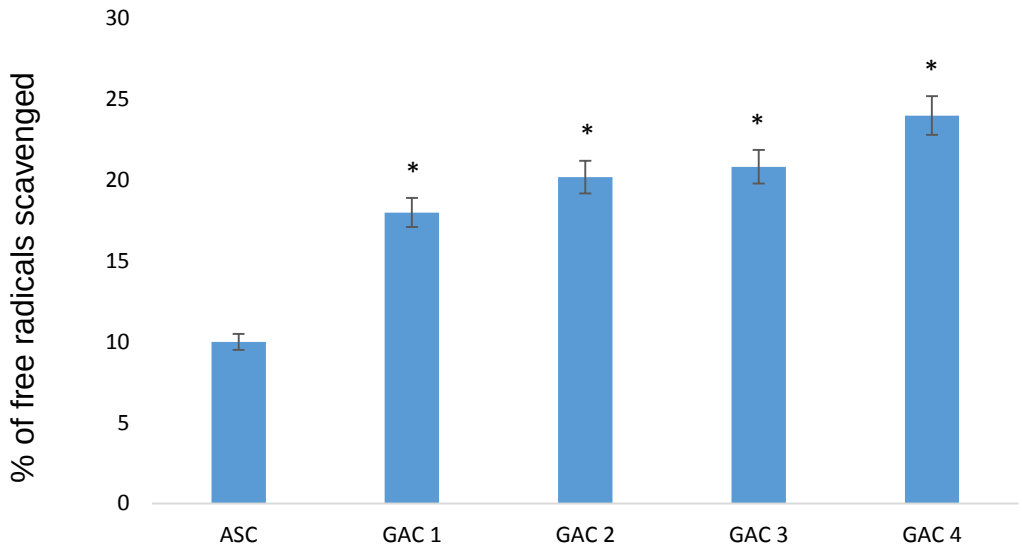

Figure 6.2. Free radical scavenging activity of GACs.
The GACs removed the free radicals more effectively than the standard ascorbic acid. The values represented are mean ± SD. ASC – ascorbic acid.*$p<0.05$, n = 3.

6.3.3 GAE and GACs enhanced SOD and POX activities

Prevention of ROS formation or their rapid removal by antioxidants protects cells from the toxic effects of ROS. In the current study, the efficacy of GAE and GACs

on SOD and POX activities of A549 cells was evaluated. The results are shown in figure 6.3 and 6.4.

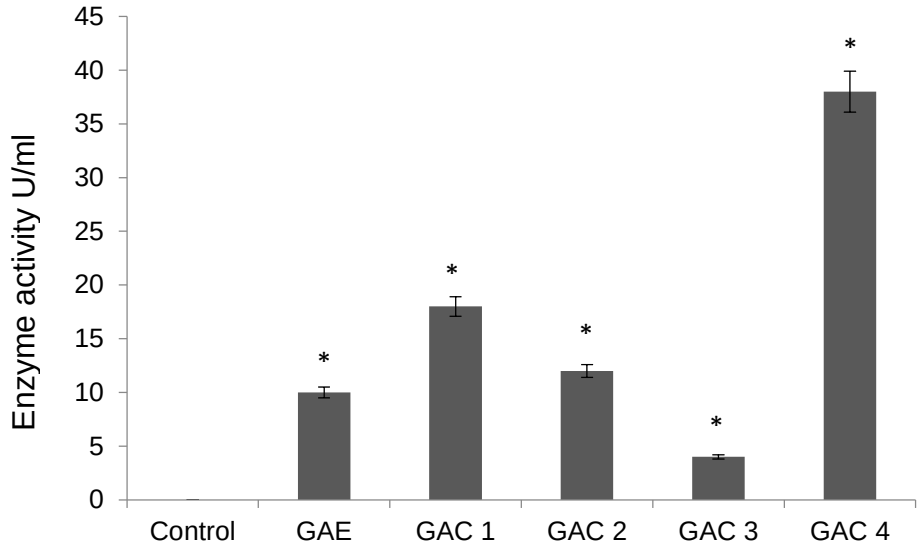

Figure 6.3 Analysis of SOD activity in A549 cells treated with GAE and GACs. Values represented are mean ± SD. n = 3, *p<0.05 for treated samples compared to untreated control.

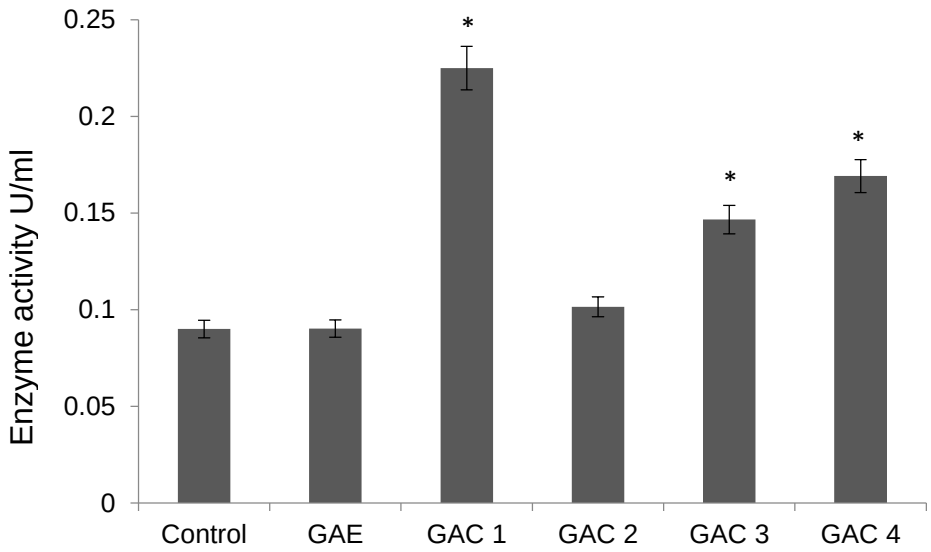

Figure 6.4 Analysis of POX activity in A549 cells treated with GAE and GACs. Values represented are mean ± SD. n = 3, *p<0.05 for treated samples compared to untreated control.

The cell lysate from the untreated cancer cells showed loss of SOD activity whereas; treatment with GAE and GACs increased the level of SOD activity in cancer cells (Figure 6.3). Among the four GACs, the GAC 4 treatment increased the SOD activity (38U/ml) followed by GACs 1, 2 and 3. Treatment with the crude extract GAE also increased the SOD activity but was comparatively lesser than the individual GAC treatment. Similarly, the POX activity was also investigated in the current study. The POX activity was significantly reduced in untreated cancer cells which increased after treatment with GACs (Figure 6.4). The GAC 1 was more effective in enhancing the POX activity followed by GACs 3, 4 and 2. Treatment with crude extract (GAE) did not have any effect on POX activity. The outcomes of the current analysis revealed that the GACs are capable of increasing the levels of antioxidant enzymes which are essential for protecting the cells from ROS induced cellular damage.

6.4 Discussion

6.4.1 *G.acerosa* exhibited antioxidant activity

In the current study, the antioxidant efficacy of the crude algal extracts was investigated by DPPH assay. The outcomes showed that all the crude extracts demonstrated high antioxidant activity than ascorbic acid. The antioxidant activity observed may be attributed to the polyphenol and flavonoid content of these extracts. Earlier studies have revealed that these phytochemicals contribute to the antioxidant efficacy of plants and herbs [172] and function as potent free radical scavengers. They possess high redox properties which can either neutralize the free radicals or degrade the peroxides [173].

In the current study, the ethyl acetate extract (GAE) exhibited strong antioxidant activity than the other extracts. Our results (Figures 4.1 and 4.2) showed that polyphenols and flavonoids were in abundance in GAE. Hence the free radical scavenging capacity of the GAE may be due to its polyphenol and flavonoid content. As a significant correlation is observed between the phenol, flavonoid concentration and the DPPH scavenging activity, the antioxidant capacity of the extracts may be contributed by these bioactives. The results of the analysis correlated with earlier studies in *G.acerosa* [14], [63] thus confirming the algal extract as a good antioxidant.

6.4.2 GAE and GACs induced SOD and POX activities

Lower activity of antioxidant enzymes is directly related to the oxidative damage induced by ROS accumulation [30]. In the present investigation, decreased activities of the antioxidant enzymes SOD and POX were observed in cancer cells which correlate with the oxidative stress in cancer. Medicinal plants and herbs are considered as the major repository of antioxidants which is contributed by the phytochemicals which have the ability to neutralize or trap the free radicals [174]. The antioxidant activity of *G.acerosa* was demonstrated in previous studies [63]. Similarly, *G.acerosa* was shown to enhance the activities of antioxidant enzymes and protected PBMCs from TCDD induced toxicity [14]. These results are in

correlation with our present findings and thus confirming the antioxidant activity of *G.acerosa*.

6.5 Conclusion

The overall outcomes revealed that the algal compounds are found to be capable of enhancing the antioxidant defense mechanism. This shows that GACs are natural antioxidants. Generally, natural antioxidants are preferred than synthetic ones to minimize the toxic effects on humans. Based on the current observations, both GAE and GACs possess high antioxidant activity and can be considered as an alternative to synthetic antioxidants.