CHAPTER 7

Discussion, Summary, Conclusion and Future Scope
7.1 DISCUSSION

UV-B radiation is a minor component of the solar spectrum reaching the Earth but is most effective in causing sunburns, aging, immune reactions and skin cancer. It reacts with photosensitizers and leads to formation of free radicals which cause various systemic diseases (Patlolla et al., 2010). DNA can absorb UV-B directly leading to formation of DNA strand breaks, thymine glycols and 8-hydroxyguanine (Johnson et al., 2008). Hence, protection from UV-B radiations is very necessary. Some of the natural plant compounds extensively studied for photoprotection are Silymarin (Svobodova and Psotova, 2003), sesamoil (Ramchandran et al., 2010), ursolic acid (Ramchandran and Prasad, 2008), ferulic acid (Prasad et al., 2007), epicatechin gallate (Huang et al., 2007) which have successfully attenuated the UV-B induced cytotoxicity, antioxidant depletion, ROS generation, DNA damage, apoptotic morphological changes, etc.

Flavonoids, a type of polyphenols possess good antioxidant potential. Ethyl acetate fractions of E. caryophylata (Clove), F. vulgare (Fennel seed) and A. esculentus (Okra) showed significant flavonoid content and antioxidant potential, hence, were studied further for its photoprotective ability. The fractions exhibited a free radical scavenging and a reducing potential as observed in DPPH, ABTS and FRAP assays respectively. Antioxidant ability of flavonoids depends on the structure and substitution pattern of hydroxyl groups i.e. 3', 4'-orthodihydroxy configuration in B ring and 4-carbonyl group in C ring and also the presence of 3-OH or 5-OH group is essential (Wojdyla et al., 2007).

Interaction of UV-B radiations with the cellular chromophores and photosensitizers leads to formation of ROS and damage to the various bio-molecules like lipids, proteins, DNA, etc. In the current study, pre-treatment of cells with ethyl acetate fractions significantly reduced the formation of ROS which reflects the free radical scavenging property of the fractions.

Endogenous antioxidant system protects against oxidative damage and comprises of various enzymes like SOD, CAT, GPx and GR and other non-enzymes like bilirubin, uric acid, etc. In the current study, UV-B induced ROS generation may have depleted the levels of endogenous antioxidant enzymes. The antioxidant enzymes are affected due to direct absorbance of UV-B radiation, interaction with
ROS or the antioxidant recycling mechanisms. Haem group absorbs UV-B radiations and decreases CAT activity; depleted SOD may be due to the formation of superoxide anion and antioxidant recycling mechanism may be the reason for decreased GPx and GR activities (Lu et al., 2010). Pre-treatment of cells with ethyl acetate fractions retained the levels of endogenous antioxidant enzymes significantly with a decrease in the levels of ROS. The fractions may be protective against diseases caused due to UV-B induced oxidative stress.

UV-B induced DNA damage was also observed in human dermal fibroblasts. Oxidative stress induced DNA damage may be due to the direct absorption of UV-B by DNA or through the formation of ROS (Shindo et al., 1994). Pre-treatment of cells with ethyl acetate fractions of all the 3 plants significantly reduced the DNA damage as seen in comet assay which may be due to the sunscreen effect exerted on DNA. They were also observed to be non-genotoxic and non-mutagenic. Protective effect of ethyl acetate fractions on UV-B induced apoptotic changes was studied by acridine orange/ ethidium bromide staining. Control cells had intact nuclei and no changes were seen in the morphology. Ethyl acetate fractions protected against UV-B induced loss of cell membrane integrity, nuclear fragmentation and chromatin condensation.

Nrf2-ARE pathway is important in cellular defence. As observed in the results, UV-B induced excessive generation of ROS leading to increase in oxidative stress which can be co-related to the increase in expression of Nrf2 and HO-1, whereas, reduction in expression of Nrf2 and HO-1 may be due to the decrease in oxidative stress in the cell after pre-treatment with ethyl acetate fractions.

In the present study, we observed that the ethyl acetate fractions of *E. caryophylata* (Clove), *F. vulgare* (Fennel seed) and *A. esculentus* (Okra) protected the cells against UV-B induced cytotoxicity, antioxidant depletion, intracellular ROS, oxidative DNA damage, apoptotic changes and also protected through Nrf2-ARE pathway.
7.2 SUMMARY

In summary, *E. caryophylata* (Clove), *F. vulgare* (Fennel seed) and *A. esculentus* (Okra) were studied for their UV-B protective potential. Crude extracts were prepared by soxhlet and cold extraction techniques. The cold alcoholic extracts exhibited a high flavonoid content and antioxidant potential. After enrichment, ethyl acetate fractions of clove, fennel seed and okra showed a better flavonoid content and antioxidant potential than cold alcoholic extracts indicating a successful enrichment. Ethyl acetate fractions of all the 3 plants protected against UV-B induced cytotoxicity, antioxidant depletion, oxidative DNA damage, apoptotic morphological changes, excessive production of intracellular ROS and over-expression of Nrf2 and HO-1. They also worked through the Nrf2-ARE pathway to confer protection against UV-B induced oxidative stress. Taken together, these findings suggest that the flavonoids from clove, fennel seed and okra could potentially be considered as UV-B protectants and can be explored further for its topical application to the area of skin requiring protection.

7.3 CONCLUSION

In conclusion, the flavonoids from *E. caryophylata*, *F. vulgare* and *A. esculentus* exhibited a significant UV-B protective potential and also worked through the Nrf2-ARE pathway to reduce oxidative stress in the HDF cells. The flavonoids from all the 3 plants can be explored for the treatment of diseases caused due to oxidative stress. They can also be developed into an herbal sunscreen formulation for topical application to the area of the skin requiring protection.

7.4 FUTURE SCOPE

- Isolation of flavonoids from the ethyl acetate fractions and studying the UV-B protective potential individually.
- A synergistic study of UV-B protective potential of the ethyl acetate fractions of *E. caryophylata*, *F. vulgare* and *A. esculentus* can be carried out.
- UV-A protective potential of the ethyl acetate fractions of *E. caryophylata*, *F. vulgare* and *A. esculentus* can be studied.