

CHAPTER V

CONCLUSIONS

It has been authorized that oxidative stress, either endogenous or exogenous, is a critical factor in the aging process. In this study, the anti-aging effects of *Artocarpus lakoocha* heartwood extract (Puag-Haad) and its active constituent oxyresveratrol were evaluated using different *in vitro* techniques. These included their effects on the fibroblast proliferation and cytotoxicity, ability to reduce or protect against oxidative stress-induced cell and DNA damages, as well as their anti-collagenase enzyme activity in comparison with other well known antioxidants commonly used in the cosmetic industry.

The results obtained in this work can be summarized as follows:

1. All six antioxidants investigated (Puag-Haad, oxyresveratrol, EGCG, pine bark extract, Trolox[®], L-ascorbic acid) had a significant proliferative effect on fibroblasts, especially at the concentrations of 25 and 50 $\mu\text{g/mL}$. At these concentrations the proliferative effect of Puag-Haad and oxyresveratrol were relatively similar to EGCG, pine bark extract and Trolox[®] but slightly higher than L-ascorbic acid. No cytotoxic effect on fibroblasts was observed for all antioxidants in the concentration range of 10 – 50 $\mu\text{g/mL}$. The optimum proliferative concentration was about 25 $\mu\text{g/mL}$.

2. The cytotoxicity of Puag-Haad and reference antioxidants was evaluated using the same MTT assay. As the antioxidant concentration was further increased from 25 to 100 and 250 $\mu\text{g/mL}$, the proliferative effect declined in all antioxidants. At 250 $\mu\text{g/mL}$ the effect became cytotoxic to the fibroblasts, with the number of viable cells significantly lower than control in the case of Puag-Haad, oxyresveratrol, EGCG and L-ascorbic acid. Pine bark extract and Trolox[®], on the other hand, did not produce significant cytotoxicity at this concentration.

3. The protective effect of Puag-Haad and reference antioxidants against H_2O_2 -induced cell damage was evaluated by exposure of the fibroblasts to the mixture of 2 mM H_2O_2 and various concentrations of antioxidants. Based on the MTT assay, all the antioxidants except L-ascorbic acid were able to provide protective effect against H_2O_2 at all concentrations studied (25, 50 and 100 $\mu\text{g/mL}$) as the number of viable fibroblasts was significantly greater than the control group, which was exposed to only H_2O_2 . The protective effect against H_2O_2 of Puag-Haad and oxyresveratrol was similar to EGCG, pine bark extract and Trolox[®], whereas L-ascorbic acid was effective only at 25 $\mu\text{g/mL}$ and appeared to potentiate the cytotoxicity of H_2O_2 at higher concentrations.

4. The protective effect of Puag-Haad and reference antioxidants against UV-A-induced cell damage was evaluated by exposure of the fibroblasts to 20 J/cm^2 of UV-A following overnight treatment with the test antioxidants. MTT test was also used to determine the number of surviving fibroblasts in each group. All the antioxidants were able to provide protective effect against UV-A in a concentration-dependent manner, with the effect most evident at 100 $\mu\text{g/mL}$, the highest concentration studied. The protective effect of Puag-Haad and oxyresveratrol was similar to EGCG, pine bark extract and Trolox[®] whereas L-ascorbic acid was the most effective against UV-A in this experiment. It is likely that the mechanisms of L-ascorbic acid and other antioxidants in protecting (or potentiating) cell damages might be different when using different oxidative stresses and experimental conditions.

5. Results from the LDH assay were different from the MTT test. All the test antioxidants at 25, 50 and 100 $\mu\text{g/mL}$ failed to reduce LDH leakage from the fibroblasts under UV-A radiation. Instead, they were found to induce LDH release in a concentration-dependent manner. At 100 $\mu\text{g/mL}$, L-ascorbic acid gave the highest LDH release (77.34%), followed by EGCG (71.60%), oxyresveratrol (61.92%), pine bark extract (61.01%), Trolox[®] (60.14%) and Puag-Haad (57.69%). The data suggested that although these antioxidants may be able to protect the cells against oxidative stress-induced damages based on the MTT test results, they may also

interact directly with the cell membrane, causing irritation and simultaneous leakage of intracellular LDH enzyme.

6. Staining of DNA by propidium iodide (PI) followed by flow cytometric analysis of fibroblasts exposed to UV-A radiation revealed that both Puag-Haad and oxyresveratrol at 25 and 50 $\mu\text{g}/\text{mL}$ could reduce the number of non-viable cells (cells in the sub G_0/G_1 phase or in the late apoptotic process) with concomitant increase in the number of viable cells when compared to control (cells exposed to UV-A without antioxidant). The results therefore agreed with those previously obtained from the MTT assays that Puag-Haad and oxyresveratrol could prevent or reduce cell damages caused by oxidative stresses in the fibroblast model.

7. Evaluation of anti-collagenase activity revealed that all the six antioxidants were capable of inhibiting collagenase type IV *in vitro*. The IC_{50} values, which are indicator of their inhibitory potency, were calculated from plots of percentage inhibition versus antioxidant concentration and statistically analyzed by ANOVA and Tukey's test. The ranking of the IC_{50} values was as follows:

EGCG \approx Pine bark < Puag-Haad < oxyresveratrol < L-ascorbic acid < Trolox[®]
 0.008 0.0219 0.0588 0.1531 1.3314 2.348 mg/mL

EGCG and pine bark extract were the most potent inhibitors, followed by Puag-Haad and oxyresveratrol. L-ascorbic acid and Trolox[®], on the other hand, demonstrated a much weaker inhibitory activity than Puag-Haad and oxyresveratrol due to their very high IC_{50} values (L-ascorbic acid, for example, was more than 20 folds weaker than Puag-Haad). Thus, apart from the proliferative effect on the fibroblasts, Puag-Haad and oxyresveratrol might increase the amount of collagen, a fibrous protein crucial for the integrity of the dermis, via inhibition of the collagenase enzyme.

8. In conclusion, the results from the present study indicated that Puag-Haad or the aqueous extract of *Artocarpus lakoocha* heartwood and its active constituent oxyresveratrol exhibited significant proliferative effect on the fibroblasts. They were able to provide protection against cellular damages caused by oxidative stresses and also possessed a good anti-collagenase activity. Their beneficial effects and mild cytotoxicity were comparable to the commercially available antioxidants. Thus, considering the above activities together with the more economical cost, Puag-Haad may have a very strong potential for use as an anti-aging agent in cosmetic industry provided that more studies are undertaken to confirm its efficacy and safety in human volunteers.