

CHAPTER V

CONCLUSIONS

In this study, oxyresveratrol was isolated from the dried aqueous extract of *Artocarpus lakoocha* heartwood (Puag-Haad) and both the extract and the active compound were further evaluated for their antioxidative/free radical scavenging activities using several in vitro systems in comparison with other well known antioxidants used in the cosmetic industry. In addition, the physical and biochemical stability of Puag-Haad in aqueous solution, with and without stabilizers were also investigated.

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

1. The extraction of oxyresveratrol, the active constituent of Puag-Haad, was successfully carried out by chromatographic method. The yield was 14.83 % w/w.
2. For the identification of the active constituent in Puag-Haad, the TLC chromatograms and NMR spectra revealed that the purified compound gave the R_f value and chemical shifts corresponding to oxyresveratrol with about 95% purity.
3. Upon standing at ambient temperature, the color of Puag-Haad solution without any antioxidant darkened to form light brown color. Addition of sodium metabisulfite, either alone or in combination with BHA and Rovisome[®] (liposome containing magnesium ascorbyl phosphate), was able to maintain the original color, which was pale yellow. However, neither BHA nor Rovisome[®] could stabilize the color of Puag-Haad solution unless sodium metabisulfite was present.
4. After 24-week storage period, the pH of Puag-Haad solution without any stabilizer slightly dropped. Similar result was found in the Puag-Haad solution with

8. Regarding the hydroxyl radical scavenging activity, Puag-Haad and oxyresveratrol appeared to have potency comparable to EGCG but slightly less potent than l-ascorbic acid and Trolox[®]. Pine bark, on the other hand, was found to be the least potent hydroxyl radical scavenger. The rankings of the IC₅₀ values are as follows:

l-ascorbic acid ≈ Trolox[®] < EGCG ≈ oxyresveratrol ≈ Puag-Haad < pine bark extract
 1.57 2.39 6.20 7.41 9.47 48.56 μg/ml

9. The ability of the test antioxidants to scavenge singlet oxygen was evaluated using the UV-induced lipid peroxidation of red blood cells in the presence of a photosensitizer (hematoporphyrin). The control group without addition of any antioxidant was found to have maximum hemolysis. Addition of the test antioxidants (except l-ascorbic acid and Trolox[®]) resulted in the reduction of % hemolysis in a concentration dependent manner. Comparison of % relative hemolysis and their IC₅₀ values showed that Puag-Haad, oxyresveratrol and pine bark extract were equally potent singlet oxygen scavengers whereas EGCG was much less effective as seen from the ranking of % hemolysis remaining after addition of the test antioxidants at 600 μg/ml as well as from the IC₅₀ values shown below:

	Pine bark extract	~ oxyresveratrol	~ Puag-Haad	< EGCG	
% hemolysis	6.20	9.05	14.96	75.02	%
IC ₅₀	0.20	0.26	0.28	0.80	mg/ml

10. Interestingly, l-ascorbic acid and Trolox[®] failed to show any antihemolytic effect and thus no singlet oxygen scavenging activity was detected at the concentration ranging from 200 to 600 μg/ml. This could be due to the pro-oxidant effect of the two vitamins at these high concentrations or some other free radicals could be generated in the systems which might counteract their scavenging activities.

11. In conclusion, the results from this study showed that Puag-Haad, which is the crude aqueous extract of *A. lakoocha* heartwood, was capable of scavenging several

reactive oxygen species including superoxide anion, hydroxyl free radical, singlet oxygen as well as DPPH, a synthetic free radical often used as a standard model. Its antioxidant potency appears to vary depending on the type of reactive species being scavenged. The extract (and its active constituent oxyresveratrol) was found to be most active against singlet oxygen when compared to other antioxidants commonly used in the cosmetic products. Considering its various antioxidative properties together with the previously reported antityrosinase activity, the inexpensive and easily available *A. lakoocha* extract or Puag-Haad has a very promising potential for use as an antioxidant for both the pharmaceutical and cosmetic applications.



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