

CHAPTER V

CONCLUSIONS

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

Part 1. Development, validation of HPLC method and determination of oxyresveratrol content in *Artocarpus lakoocha* heartwood extract (Puag-Haad)

1. The HPLC method for analysis of oxyresveratrol, the active constituent of the dried aqueous extract of *Artocarpus lakoocha* heartwood (Puag-Haad) has been developed in this study. The column was Luna Phenomenex[®] C18 (2) (5 μ m, 250 x 4.6 mm) and the mobile phase was methanol : water (40:60) with the flow rate of 1 ml/min. The UV detector was set at 329 nm. The retention times for oxyresveratrol, resveratrol (its related substance), and the internal standard (furazolidone) were 11.49, 19.45, 5.51 min, respectively.
2. The method showed good specificity, linearity, accuracy and precision and was subsequently used for the assay of oxyresveratrol content in Puag-Haad powder as well as its stability in solutions.
3. The oxyresveratrol content of Puag-Haad powder (Lot no.25/10/04) was found to be 85.11 ± 0.25 % w/w.

Part 2. Stability evaluation of different aqueous solutions of Puag-Haad

2.1 Stability evaluation of Puag-Haad in three buffer systems

1. After storage at 45 °C for 4 weeks, Puag-Haad solution in citrate buffer pH 4.0 to 5.5 was found to provide the optimum stability from the both physical and chemical aspects. The solution did not change its color along the storage time and gave the highest content of oxyresveratrol (more than 90% remaining) after storage at this condition.

2. Puag-Haad solution in phosphate buffer at either pH 5.5 or 7.0 failed to provide protection against chemical degradation and discoloration. It even promoted

the degradation especially at higher pH. This could be due to the general base catalysis exerted by the phosphate species.

3. The pure Puag-Haad solution without buffer showed a slight drop in pH (0.59 unit) whereas addition of either phosphate or citrate buffer was able to stabilize the pH of Puag-Haad solution due to their buffer capacity.

4. Citrate buffer pH 5.5 was selected as a buffer medium of Puag-Haad solution for further stability evaluation due to its closeness to skin pH as well as its good stabilizing effects on pH, color and content of oxyresveratrol.

2.2 Preliminary stability evaluation of aqueous solutions of Puag-Haad in the presence of various antioxidants

1. After storage at 45 °C for 4 weeks in 20% v/v propylene glycol and 80% v/v citrate buffer pH 5.5, addition of 0.10% w/v sodium metabisulfite was able to maintain the original color of Puag-Haad solution, which was pale yellow. However, addition of either 0.02% BHA, 0.10% propyl gallate or 0.05% EDTA could not stabilize the color of Puag-Haad solutions unless sodium metabisulfite was also present.

2. Despite the ability to stabilize color, addition of sodium metabisulfite especially at high concentrations (0.10 - 0.15%) gave a large drop in the content of oxyresveratrol in Puag-Haad solution. This could be due to direct interactions of sodium metabisulfite with oxyresveratrol, which may have resulted in its rapid degradation. However, more studies are needed to clarify this observation.

3. Based on the 4 week-stability evaluation, the antioxidant that could provide the highest content of oxyresveratrol appeared to be either 0.02% BHA alone or 0.05% EDTA alone in citrate buffer pH 5.5. However, concomitant addition of sodium metabisulfite, although detrimental to oxyresveratrol, may be necessary at lower concentrations (e.g. 0.01 – 0.02%) so as to achieve a good color stability while maintaining an acceptable content of oxyresveratrol in Puag-Haad solution.

2.3 Long term stability study of aqueous solutions of Puag-Haad in the presence of various antioxidants

1. After storage at 45 °C for 12 weeks, the best color stabilization could be achieved by addition of 0.02% sodium metabisulfite (either alone or in combination

with 0.02% BHA) or simply by nitrogen purge. EDTA alone provided the worst color stabilization. Similar pattern was observed at 30 °C for 24 weeks.

2. Based on the data at 45 °C, the optimum antioxidant system which could provide sufficient chemical stability appeared to be 0.02% BHA alone, with percent oxyresveratrol remaining of 63.77% after 12 weeks.

3. Based on the data at 30 °C, the optimum antioxidant system may include not only 0.02% BHA but a combination of 0.02% BHA and 0.02% sodium metabisulfite, in which only a slight drop in percent oxyresveratrol (81.92% remaining after 24 weeks) and better color stability was achieved. Also, purging the solution with nitrogen could greatly enhance the chemical stability without addition of any antioxidants.

Part 3. Evaluation of anti-wrinkle efficacy of Puag-Haad solutions in human volunteers

1. The initial values of circular and mean roughness parameters of the subjects' cheek area (either left or right side) measured at week 0 were similar among the four groups ($P > 0.05$), indicating the homogeneity and balanced distribution of the subjects for the study.

2. The baseline values of circular and mean roughness of the subjects was also monitored for four weeks prior to start of the experiment. There was no significant difference in the baseline values due to time effect (week -4, -2 and 0) or application site (left and right cheeks) in all groups ($P > 0.05$).

3. The roughness values of the propylene glycol-treated cheek (PG-self control) appeared to increase slightly from the initial time (week 0) in all groups, indicating that the solvent alone might increase the skin roughness. This was probably due to the skin-dehydrating effect of propylene glycol. The average circular and mean roughness of all the antioxidant-treated cheeks did not increase with application time and in some groups (such as 0.10% Puag-Haad and 0.10% EGCG solutions) even appeared to decrease slightly. However, randomized block ANOVA did not show significant change with application time in both the control and antioxidant-treated sides ($P > 0.05$).

4. Application of 0.10% Puag-Haad solution appeared to be the most effective, with the roughness values (both circular and mean) becoming significantly

lower than its self-control after only 2 weeks of application and remained significant until the last week (week 8) of the study ($P < 0.05$). This was followed by 0.25% Puag-Haad and 0.10% EGCG, which demonstrated similar anti-wrinkle efficacy at the last week (week 8) of application. 0.10% vitamin C, on the other hand, appeared to be the least effective with significant anti-wrinkle effect observed only at week 4 for circular roughness and not at all for mean roughness.

Part 4. Formulation of anti-wrinkle lotions containing Puag-Haad

Fifteen oil-in-water emulsion formulations containing 0.10% w/v Puag-Haad were prepared and tested under accelerated conditions. Formulation no. 12 was selected for study in human volunteers due to its stability after 6 heat-cool cycles in term of physical appearances, viscosity, pH and skin feel. The formula contained 0.02% BHA as antioxidant and was buffered with citrate buffer pH 5.5.

Part 5. Evaluation of anti-wrinkle efficacy of lotions containing Puag-Haad in human volunteers

1. Lotion containing 0.10% Puag-Haad gave a more rapid anti-wrinkle effect, with the values of both circular and mean roughness become significantly lower than the lotion base (self-control) after 6 weeks of application ($P < 0.05$), whereas 0.10% EGCG lotion gave significant effect in both parameters after 8 weeks.

2. At week 8, 0.10% Puag-Haad lotion gave % improvement in skin wrinkles over self-control of 5.93 and 5.78% for circular and mean roughness, respectively, whereas 0.10% EGCG lotion showed only 5.64 and 2.11% improvement. However, no significant difference was detected in the values of % wrinkle improvement (both circular and mean roughness) between the two lotions as analyzed by independent t-test ($P > 0.05$).

3. 0.10% Puag-Haad lotion also provided skin whitening effect, with the values of cheek skin melanin become significantly lower than the self-control lotion base after 4 weeks of application ($P < 0.05$). 0.10% EGCG lotion, on the other hand, did not produce any whitening effect during the 8-week study period.

4. Measurements of skin elasticity using Cutometer failed to detect any improvement for both the Puag-Haad and EGCG lotions. This could be due to

difficulties of the measuring techniques in addition to the sensitivity of the instrument, which might not be adequate to detect a small change in elasticity parameter.

5. Measurements of skin capacitance, an indicator of skin moisture content, revealed that both the Puag-Haad and EGCG lotions were able to significantly increase the values ($P < 0.05$). This was mainly due to the moisturizing effect of the lotion base as similar improvement was also observed in the self-control cheeks.

3. The lotion formulation used in this study was well accepted by the subjects with respect to ease of application, speed of absorption, stickiness and overall likings. All the subjects completed the study without development of irritation or other skin hypersensitivity.

4. The results from the individual subjects' self-evaluation (blinded assessment) with respect to the effect of lotion on the skin attributes such as skin roughness and elasticity were difficult to interpret and did not correlate with the data obtained from the instruments. This was due to lack of training before completing the questionnaire as well as the nature of the assessment method, which was highly subjective.

5. The data also suggested that evaluation of anti-aging/anti-wrinkle effect needs highly sensitive instruments which are capable of objectively quantitating slight changes in the skin conditions and that a clinically significant, visually noticeable effect may be more elusive or difficult to achieve.

In conclusion, the results in this present work have demonstrated that the aqueous extract of *Artocarpus lakoocha* heartwood or Puag-Haad had an anti-wrinkle activity. Its effect was more rapid and superior to EGCG and vitamin C at the same concentration. The extract also possesses a skin whitening effect and is available at a much more economical cost. Thus, Puag-Haad has a very promising potential for use as a multi-functional antioxidant, anti-wrinkle and skin-whitening agent in cosmetic preparations