CHAPTER V

CONCLUSION AND RECOMMENDED FUTURE EXPERIMENTS

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

Part 1. Evaluation of skin whitening efficacy of Puag-Haad and Haadnun in guinea pigs.

The dried aqueous extracts of *Artocarpus lakoocha* heartwood (Puag-Haad) and *Artocarpus gomezianus* root (Haadnun) were investigated in guinea pig model for their *in vivo* skin whitening activity. The dried extract of both plants were dissolved in propylene glycol at 0.5% for Puag-Haad and 3% for Haadnun. Their whitening effects were then compared with 3% kojic acid solution in propylene glycol (positive control) and pure propylene glycol (negative control) using a parallel study design (6-7 guinea pigs in each group).

1. After daily application of each substance on the shaved area of the guinea pig back skin, the melanin (M) and erythema (E) values were measured at 2 and 4 weeks using Mexameter MX 16. The results showed that 0.5% Puag-Haad was the most effective whitening agent among the four groups, giving the overall % whitening after 4 week-application of 7.59%, which was significantly greater than 3% kojic acid (5.38%) and 3% Haadnun (5.27%) and propylene glycol (3.26%) ($P < 0.05$).

2. Increasing the concentration of Puag-Haad from 0.5% to 1.0% resulted in a decrease in whitening activity, probably due to some unknown feedback mechanism(s). Increasing the concentration of Haadnun from 3% to 5%, on the other hand, resulted in similar whitening activity, indicating the possible saturation of enzyme inhibition.

3. No significant differences were observed in the E values among Puag-Haad, Haadnun-, kojic acid- and propylene glycol-treated guinea pigs, suggesting that all the compounds did not cause significant erythema during the 4-week study.

Part 2. Evaluation of skin whitening efficacy of Puag-Haad in human volunteers.

Eighty female volunteers (initial melanin values 477 - 552) participated in a parallel study with self-control. They were divided into four groups of 20 each. Group A received 0.50% Puag-Haad solution in propylene glycol on one arm and pure propylene glycol (self-control) on the other. Similar methodology was also employed for subjects in groups B, C and D, who respectively received solutions of 0.25% Puag-Haad, 0.25% licorice extract and 3% kojic acid on one arm and pure propylene glycol on the other remaining arm. Each subject was monitored for baseline melanin (M) values for 2 weeks before daily application of either A, B, C or D and self-control propylene glycol.

1. The initial M values measured at week 0 (immediately before application) were similar among the four groups, indicating the homogeneity and balanced distribution of the subjects with respect to their initial melanin contents within each group.

2. After application of the substances and self-control, each subject was monitored for the M and E values using Mexameter at two-week intervals for 12 weeks. Efficacy of the whitening agent was detected when paired student' t- test within each group showed significant difference ($P < 0.05$) in % whitening relative to the initial value between the arms treated with the substance (A, B, C or D) and the arms treated with propylene glycol (self-control).

3. Paired t-test results revealed that 0.25% Puag-Haad was the most effective agent with respect to the rate of whitening, giving the shortest onset time to detect significant whitening at only 4 weeks after application, followed by 3% kojic acid which started to show significant whitening after 8 weeks. On the other hand, both 0.25% licorice and 0.50% Puag-Haad showed significant whitening after 10-week application.

4. The reasons as to the slower onset of 0.5% Puag-Haad than 0.25% concentration are not clearly known. However, the data agreed with the results from the guinea pig study in that certain optimal concentration range for Puag-Haad may exist, which needs to be established in more details in the future.

5. One-way ANOVA was also applied to compare the extent of whitening among the four groups after 10 and 12 weeks, at which time all groups had shown significant whitening effect over their respective self-controls. The results showed no significant difference in the whitening extent ($P > 0.05$). Nevertheless, the value for 0.25% Puag-Haad tended to be greater than the other treatment groups.

6. All the four substances appeared to have some erythema-reducing effect compared to their self-control propylene glycol (paired t-test). This observation could be due to their protective effect (e.g. anti-inflammatory activities) against skin irritants such as UV light or certain soaps.

7. Visual observation of subjects' treated skin areas was also made. Dryness of skin with some scaling of epidermis was the most commonly observed symptom, due mainly to the hygroscopic effect of propylene glycol. Only two subjects (one each in group A and D) experienced visible skin rash and voluntarily withdrew from the study after 4 weeks. Two more dropped out later from personal reasons not related to side effects. The remaining subjects completed the study and apparently showed good tolerance of the applied compounds.

Part 3. Stability evaluation of Puag-Haad aqueous solution.

Solutions of 0.25% Puag-Haad dissolved in 20% propylene glycol/80% water, with and without different antioxidants, were tested for physical and biochemical stability upon storage at room temperature and at 45 °C.

1. The starting color of all solutions was clear having similar pale yellow color. However, upon standing at room temperature for 24 weeks, the color of Puag-Haad without any antioxidant darkened to form dark brown color. Addition of sodium metabisulfite, either alone or in combination with BHA and/or EDTA, was able to maintain the original color. However, BHA, EDTA and their combination failed to stabilize the color of Puag-Haad solution. Similar results were observed at 45 °C.

2. The pH of Puag-Haad slightly dropped upon storage. The presence of sodium metabisulfite, either alone or in combination with BHA and/or EDTA, caused a further drop in pH values. This was due mainly to the degradation of sodium metabisulfite to form sulfate and hydronium ions since pure sodium metabisulfite solution also showed a similar decrease in pH. On the other hand, EDTA was found to stabilize the pH of Puag-Haad probably due to its chelating action whereas BHA had no effect on Puag-Haad pH.

3. Biochemical stability was determined by measuring % tyrosinase inhibitory activity of each solution. Without antioxidants, the inhibitory activity of Puag-Haad decreased substantially by 50% and 61% upon 24-week storage at room temperature and at 45 $^{\circ}$C, respectively. Addition of different antioxidants was found to stabilize the activity to a varying extent. The best antioxidant combination that provided optimum protection against loss in % inhibition and change in color was the mixture of sodium metabisulfite and BHA. Nevertheless, more studies are needed to determine the most appropriate concentration of each antioxidant to maximize its protective effect. Slowing the self-degradation of sodium metabisulfite was another approach that should be attempted to achieve maximum stabilization of Puag-Haad.

In conclusion, the results in the present work have shown that Puag-Haad or the dried aqueous extract of *Artocarpus lakoocha* heartwood possessed potent tyrosinase inhibitory activity both *in vitro* and *in vivo*, resulting in a rapid and significant skin whitening effect which was superior to licorice extract or kojic acid. Apart from the high efficacy, the extract also demonstrated low skin irritation potential and is available at a much more economical cost. These favorable attributes all point to the promising potential of Puag-Haad as a novel whitening agent for commercial application in cosmetic and/or dermatological products industry.