CHAPTER V

CONCLUSION

1. Evaluation of skin whitening efficacy of aqueous extract of Artocarpus lakoocha heartwood (Puag-Haad), niacinamide, lactic acid, and their combinations in guinea pigs

Three whitening agents (0.25% Puag-Haad, 1% niacinamide and 1% lactic acid) and their combinations (Puag-Haad + niacinamide and Puag-Haad + niacinamide + lactic acid) were tested for their *in vivo* whitening activity in guinea pigs. Following UVB irradiation, the five treatment solutions in propylene glycol were daily applied to the back of guinea pigs for 4 weeks. The animals were monitored for melanin reduction and change in erythema using Mexameter. The results can be summarized as follows:

1). The test solutions containing niacinamide alone (B) and in combination with Puag-Haad (D) appeared to show the fastest whitening activity, with significant effect over their self-controls detected as early as 1 week after application. Group A (Puag-Haad) and group E (Puag-Haad + niacinamide + lactic acid) were the next fastest group, showing significant whitening at week 2 and onward. Group C (lactic acid alone) gave the slowest onset of significant activity observed at week 3 after application.

2). ANOVA results on the extent of melanin reduction at the last week suggested that, once effective, all the test substances whether alone or in combination, did not significantly differ in their whitening activities. However, subtle differences in the whitening activity among different groups appeared to exist. The double combination containing 0.25% Puag-Haad and 1% niacinamide (group D) gave the highest whitening extent, with the mean melanin reduction of 34.33. The relative ranking was Puag-Haad + niacinamide (34.33) > Puag-Haad + niacinamide + lactic acid (28.72) > lactic acid (23.68) ~ niacinamide (23.5) > Puag-Haad (18.87). Thus,

the combination of two or three whitening agents might have a tendency to produce a further increase in whitening effect than a single component.

3.) All the five treatments at the concentrations employed in this study were well tolerated by the guinea pigs as judged from the erythema data and visual observations. No serious signs of skin disorders were detected such as contact dermatitis and skin lesions.

2. Evaluation of skin whitening efficacy of aqueous extract of Artocarpus lakoocha heartwood (Puag-Haad), niacinamide, tranexamic acid, and their combinations in human volunteers

To confirm the animal study, a study was conducted in 84 female human subjects. They were divided into six groups of 14 volunteers. Five test lotions were prepared using the same lotion base and designated as A, B, C, D, and E. Lotions A, B and C respectively contained 0.25% Puag-Haad, 1% niacinamide, and 2.5% tranexamic acid. Lotion D contained Puag-Haad in combination with niacinamide at the same concentration whereas lotion E contained Puag-Haad plus tranexamic acid. A commercial product (designated as F) was used as a reference whitening lotion. Each subject separately applied one of the test lotions on one arm and the control lotion base (no whitening agents) on the other arm twice daily for 12 weeks. The results can be summarized as follows:

1). Subjects treated with lotions A, B, C, D and F demonstrated the same onset of significant melanin reduction, which occurred at the last week (week 12). However, the combination of Puag-Haad and tranexamic acid (group E) was not effective at all weeks. The extent of the melanin reduction at 12^{th} week was 4.89 for 0.25% Puag-Haad (A), 4.44 for 1% niacinamide (B), 4.56 for 2.5% tranexamic acid (C), 4.93 for 0.25% Puag-Haad +1% niacinamide (D), and 4.04 for the commercial product (F). These values were small and did not differ significantly after ANOVA, suggesting the same whitening efficacy.

2). Small but significant erythema reduction was also found in subjects of group A, B, C, and D at 12^{th} week but the values were not different after ANOVA. The reasons as to the erythema-reducing effect of these products were not clearly known. Lotions E and F, on the other hand, failed to show any significant changes on erythema. All the test and control lotions were well tolerated by most subjects without serious skin disorders. Only one subject dropped out as a result of hypersensitivity, which occurred in both arms.

3). The slow onset and low extent of whitening efficacy was attributed to the poor subject compliance as the mean melanin values in all groups remained relatively constant from week 0 through week 10 and abruptly dropped during week 10 – 12 after a warning statement to cooperate had been issued.

3. Evaluation of skin whitening efficacy of aqueous extract of Artocarpus lakoocha heartwood (Puag-Haad), niacinamide, tranexamic acid, and their combinations in human volunteers. The extra-experiment conducted at Nakornratchasima (Korat)

. In order to obtain a more accurate and explainable results, another set of human study was conducted at Nakorn Ratchasima facility. The subjects were 42 female volunteers who were under custody at the Baan Metta House, which belongs to the Ministry of Labour and Public Welfare. The protocol was exactly the same as in the previous human study, with the exception that application of the lotions was performed by licensed nurses to ensure absolute subject's compliance and full adherence to the protocol.

1). After 8 weeks of application, the data showed that the fastest onset of significant melanin reduction occurred at week 6 after application of lotions containing 2.5% tranexamic acid (group C), 0.25%Puag-Haad + 1% niacinamide (group D), and the commercial product (group F). The other groups, i.e. 0.25% Puag-Haad alone (A) and 1% niacinamide alone (B), began to demonstrate statistical difference at week 8.

2). ANOVA also revealed that the whitening efficacy at week 8 was similar among the five effective groups (A, B, C, D and F). Nevertheless, subtle differences were detected. The ranking in the extent of melanin reduction at week 8 was D (Puag-Haad + niacinamide, 17.29) > A (Puag-Haad, 13.97) ~ B (niacinamide, 13.94) > F (commercial product, 12.20) > C (tranexamic acid, 10.29).

3). On the other hand, group E (Puag-Haad + tranexamic acid), was not effective at all weeks which was similar to the result of the previous human study. More studies are needed to verify the whitening efficacy of this double combination or to proof otherwise, i.e., Puag-Haad and tranexamic acid may be able to counteract each other's activity.

4). Evaluation of the erythema data did not detect any significant reduction from control in all groups (except group D at week 4) although the erythema values of the treated arms tended to be slightly lower than the control arms at week 6 and 8. This indicated that all the six lotions had negligible erythema-reducing effect. Only two subjects withdrew from the study as a result of hypersensitivity to niacinamide and component of the lotion base. The remaining 40 subjects completed the 8-week study without noticeable skin irritation.

In conclusion, all the test lotions (except lotion E) were effective in significantly reducing the melanin value in the subject's upper arms. The lotion containing only 0.25% Puag-Haad (A), a novel whitening agent from a commonly found plant *A. lakoocha*, was capable of producing significant whitening activity in humans equivalent to other existing, more expensive whitening agents. Furthermore, its combination with 1% niacinamide (lotion D) further increased the whitening extent with shorter onset time, which agreed with the results obtained from guinea pigs. The incidence of skin irritation was low and most subjects well tolerated the test preparations. Thus, it is possible to formulate Puag-Haad in an emulsion-type lotion base, either alone or in combination with other whitening agents, to produce a safe and effective whitening product for cosmetic and medical applications.