CHAPTER 5

CONCLUSION

In this study, 26 Thai plants were selected based on their usage in hair caring and hair loss treatment, their phytochemical constituents. In addition, these selected plants have not been studied or reported about the 5α-reductase inhibitory activity or hair growth promoting activity before. For the extraction of active substances from plants, ethyl alcohol was used. The yields of extraction were varied from 1.9 to 21.63 % of their fresh weight. Among these plants, *P. emblica* gave the highest yield of extraction and *I. aquatica* gave the lowest yield.

For quality controlling of the plant extracts, total phenolic content was used as a simple marker. Folin-Ciocaltaeu method was used for determination of total phenolic content, by using gallic acid as a standard. Total phenolic content of the selected plants were varied from less than 10 to 450.03 mg gallic acid equivalent per g extract. Among these plants, highest total phenolic content was found in *P. emblica*.

For assessment of 5α-reductase inhibitory activity, the microsomal enzyme was prepared from rat's liver. Finasteride was used as a standard. IC_{50} of finasteride was 0.394 μM. Enzyme inhibitory activity of plants were reported in the term of finasteride equivalent 5α-reductase inhibitory activity or FEA value. FEA values of tested plants were varied from 5.56 to 24.67 mg finasteride equivalent per g extract. *C. tinctorius* or safflower was the most potent 5α-reductase inhibitor.

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Hair growth promoting activity of *C. tinctorius* (safflower), *P. emblica* (emblica) and *C. ternatea* (butterfly pea) were further tested in the C57BL/6 mice model using minoxidil as a positive control. The result showed that extract of safflower could promote hair growth better than butterfly pea, emblica and minoxidil, respectively. The mice received safflower extract have more hair follicle in their dorsal skin than any other tested compounds ($p < 0.05$). There was a strong relationship between FEA value and hair growth promoting activity (in a term of Hair growth score) of the plant extract at day 14 of the treatment ($r = 0.719$). There also was a strong relationship between FEA value and hair follicle number ($r = 0.766$).

In order to deliver the active substances from safflower extract to act at the hair follicle, NLC was used as the carrier. Hot high pressure homogenization technique was used to prepare NLC. Monostearin was selected as the wax matrix. The formula of NLC with desirable properties was as followed:

Rx	% w/w
Monostearin	7
Medium chain triglycerides	3
Brij-L4	1
Span 60	9.5
Tween 60	2
Pluronic F-68	2
Sodium cocoylisethionate	0.7
DMDM hydantoin	0.2
DI water	74.6

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This composition was tested for stability, particle size, size distribution, zeta potential, and crystallinity. The average size of this NLC was 106.6 ± 1.1 nm, polydispersity index was 0.225 ± 0.005, and zeta potential was -44.5 ± 1.8 mV. The results from DSC and XRD suggested that this NLC occurred as an amorphous system.

Five concentrations of safflower extract; ranging from 0.05 to 1 % w/w were studied for an entrapment into the wax matrix. The results showed that the viscosity of the preparation was increased by an increasing of concentration of the extract. The particle size and size distribution of entrapped formulae were not difference from that of unentrapped NLC.

In order to determine the entrapment efficacy of the preparations, simple isocratic HPLC-UV condition was developed and validated. Safflower yellow which is the predominant phytochemical in safflower florets, was used as a phytochemical marker. The validated conditions used 60% methanol as a mobile phase, column used was C-18 (250 × 4.6 mm, with particle 5 μm) with controlled temperature at 40 °C. Analyte was assessed by UV absorbance at 401 nm. Indirect method was used in an assessment of entrapment efficacy. The results showed that the one with the lowest concentration (0.05 % by weight) showed the highest entrapment efficacy. This formula was developed into three different dosage forms, which include hydrogel, nanosuspension, and o/w emulsion. The nanosuspension provided the greatest characteristics on physical appearance and stability.

Hair growth promoting efficacy of hair lotion was tested by using the C57BL/6 mice model. The results suggested that developed hair lotion proved the

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better hair growth promoting activity than minoxidil. None of any irritation signs occurred in the mice received the hair lotion.

The further study on hair growth promoting efficacy of this hair lotion in healthy human volunteer should be conducted to confirm the efficacy results obtained from mice model.

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