CHAPTER V

CONCLUSIONS

In this study, the freeze-dried water extract and the methanol extract of *Raphanus sativus* L. fresh roots were prepared and analyzed for their contents of total phenolic compounds, total flavonoids and L-ascorbic acid (vitamin C). The two extracts were subsequently evaluated for their anti-tyrosinase and antioxidant/free radical scavenging activities using several *in vitro* systems in comparison with other anti-tyrosinases and antioxidants commonly used in the cosmetic industry. In addition, the cytotoxicity to normal human dermal fibroblasts of the two extracts were also investigated.

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

1. The yields of the freeze-dried water extract and the methanol extract were 4.21 and 2.59 % w/w, respectively, based on the weight of peeled fresh root.

2. The contents of total phenolics, total flavonoids and vitamin C found in the extracts were 10.09 ± 0.07, 0.51 ± 0.007 and 24.11 ± 2.29 μg per one mg of freeze-dried water extract and were 6.59 ± 0.05, 0.33 ± 0.004 and 8.28 ± 0.20 μg per one mg of methanol extract, respectively.

3. The anti-tyrosinase activity of both extracts was determined by measuring the inhibitory effect of each extract on mushroom tyrosinase and comparing the result with the reference anti-tyrosinases (L-ascorbic acid and licorice extract). Both the freeze-dried water extract and methanol extract displayed lower inhibitory potency than the two reference anti-tyrosinases as judged from their higher IC_{50} values shown below:

licorice extract	> L-ascorbic acid	> freeze-dried water extract	> methanol extract
1.23 μg/ml	73.57 μg/ml	3.09 mg/ml	9.62 mg/ml

4. Chemical stability of both extracts was also determined based on their DPPH free radical scavenging activity. After 3-month storage at -20 °C in a desiccator, the values of % DPPH inhibition of the freeze-dried water extract and the methanol extract were not different from the initial values (0 month) at all concentrations tested. The average IC_{50} values of each extract are as shown below:

Freeze-dried water extract at 0, 1 and 3-month storage

0.643 ~ 0.648 ~ 0.643 mg/ml

0 1 3 month

Methanol extract at 0, 1 and 3-month storage

1.248 ~ 1.238 ~ 1.236 mg/ml

0 1 3 month

Thus, the two extracts were considered to be stable for at least 3 months, during which further evaluation of the extracts could be performed without interferences due to changes in the chemical stability.

5. The antioxidant/free radical scavenging activities of both freeze-dried water extract and methanol extract were determined using various tests and compared with two reference antioxidants (L-ascorbic acid and Trolox®). In DPPH assay, both the freeze-dried water extract and the methanol extract gave a lower antioxidant activity against DPPH than the two reference antioxidants as judged from their higher IC_{50} values shown below:

L-ascorbic acid > Trolox® >freeze-dried water extract > methanol extract

3.60 µg/ml 6.92 µg/ml 0.643 mg/ml 1.248 mg/ml

The freeze-dried water extract also appeared to be a more potent DPPH scavenger than the methanol extract as the IC_{50} value of the former was about half of the latter.

6. The superoxide anion scavenging property of the two extracts

was evaluated using the riboflavin-photo-oxidation method. Similar to DPPH test, the methanol extract showed less superoxide anion free radical inhibition than the freeze-dried water extract and other reference antioxidants with the following ranking based on IC_{50} values.

Trolox®	L-ascorbic acid	freeze-dried water extract	methanol extract
52.08 µg/ml	78.96 µg/ml	4.20 mg/ml	6.28 mg/ml

7. The ability of the test antioxidants to scavenge singlet oxygen was also evaluated using sodium hypochloride and hydrogen peroxide ($NaOCl/H_2O_2$) method. Similar to the previous findings, both the freeze-dried water extract and the methanol extract were much less potent than Trolox® and L-ascorbic acid. Also, the methanol extract appeared to be a weaker singlet oxygen scavenger than the freeze-dried extract. The rankings of the ability based on the IC_{50} values are as follows:

L-ascorbic acid	Trolox®	freeze-dried water extract	methanol extract
79.59 µg/ml	86.11 µg/ml	1.42 mg/ml	2.40 mg/ml

8. The observed ability of the two extracts to inhibit tyrosinase as well as their scavenging properties of many reactive oxygen species may result from the presence of L-ascorbic acid and several phenolic components within the extracts. Results also showed that the more potent freeze-dried water extract contained L-ascorbic acid, total phenolics and total flavonoids to a greater extent than the methanol extract.

9. The cytotoxicity of the freeze-dried water extract and the methanol extract against normal human dermal fibroblast cell line was determined by LDH assay. The freeze-dried water extract produced greater LDH leakage than the methanol extract, particularly at the concentration higher than 0.25 mg/ml. Results also showed that solutions of L-ascorbic acid and sinapic acid (representative of total phenolic components) could induce some leakage of LDH from the cells. Thus, the membrane-interfering activity of the freeze-dried water extract could be due to the presence of L-ascorbic acid and total phenolic compounds in greater amount than the

methanol extract. However, some other unknown components of the extracts could as well contribute to their total cytotoxic effect.

10. In conclusion, the results from this study revealed that the freeze-dried water extract, which is the crude aqueous extract of *R. sativus* fresh root and the methanol extract, which is the crude methanol extract of *R. sativus* fresh root, were capable of inhibiting tyrosinase activity and scavenging several reactive oxygen species including superoxide anion, singlet oxygen and DPPH. The freeze-dried water extract appeared to be more potent than the methanol extract with respect to both the anti-tyrosinase and antioxidant activities. This could be due to the differences in the contents of several active ingredients found in the two extracts, especially the total phenolic components (as represented by sinapic acid equivalent), total flavonoids (as represented by quercetin equivalent) and vitamin C. In addition, both extracts were considered to have a mild cytotoxic effect despite the relatively high concentrations used in the study.

Thus, judging from the multiple anti-oxidative properties, together with its mild anti-tyrosinase activity, the inexpensive and easily available *R. sativus* root extract, especially the freeze-dried aqueous extract, has a very promising potential for use as an antioxidant and anti-tyrosinase for both the pharmaceutical and cosmetic applications.