CHAPTER V

CONCLUSIONS

As skin is increasingly exposed to UV radiation, the risk of photo-oxidative damage to skin with long term detrimental effects such as wrinkles, fine lines, loss of skin tone, hyperpigmentation and skin cancer also increases. In this study, spray-dried *P. emblica* powder from fruit juice was extracted using three kinds of solvents and two different extraction processes to give five extracts including ethyl acetate, acetone (successive), acetone (direct), ethanol (successive) and ethanol (direct) extracts. Both spray-dried *P. emblica* and all of its solvent extracts were further evaluated for their antioxidant, anti-collagenase and anti-tyrosinase activities using several *in vitro* systems in comparison with its commercial product and other well-known antioxidant, anti-collagenase and anti-tyrosinase agents. In addition, preliminary evaluation of the stability of *P. emblica* extracts was also performed. The results obtained in this work can be summarized as follows:

1. The extraction of spray-dried *P. emblica* was successfully carried out using soxhlet extractor to provide ethyl acetate, acetone (successive), acetone (direct), ethanol (successive) and ethanol (direct) extracts with % yield of 4.96, 10.38, 9.79, 14.32 and 14.00% w/w.

2. From, the % yield results, both direct and successive extracts from the same solvent provided similar % yield values. Also, both ethanol (direct and successive) extracts of *P. emblica* gave the highest % yield, followed by acetone (direct and successive) and ethyl acetate extracts. Comparison of percentage yields suggested that the majority of the components in the spray-dried powder, which was derived from the aqueous fruit juice, were relatively polar and, thus, could be extracted by ethanol, the most polar solvent used, in highest quantities. However, the chemical components of each extract, which are likely to differ among various extracts should be further characterized in detail.

3. According to the antioxidant activity of *P. emblica* extracts, their H-donating and hydroxyl radical scavenging activities were investigated. The H-donating activity of spray-dried *P. emblica* and its various solvent extracts were determined using DPPH method in comparison with commercial *P. emblica* extract and other reference antioxidants. It can be concluded that all of *P. emblica* extracts demonstrated strong DPPH scavenging activity, but their effects were significantly lower than EGCG, l-ascorbic acid and Trolox®. The spray-dried *P. emblica*, acetone (both direct and successive) and ethanol (both direct and successive) extracts exhibited higher DPPH scavenging activity (lower IC_{50} values) than commercial *P. emblica* extract, whereas ethyl acetate extract provided the lowest DPPH scavenging activity as judged from its highest IC_{50} value. The ranking in an increasing order and grouping of the different antioxidants with respect to their IC_{50} values against DPPH radical, are as follows:

EGCG < l-ascorbic acid < Trolox® <

1.52 2.37 3.57 μg/mL

acetone (direct) ≈ ethanol (direct) ≈ ethanol (successive) < acetone (successive) <

4.43 4.62 4.63 5.00 μg/mL

spray-dried *P. emblica* < commercial *P. emblica* < ethyl acetate

6.29 6.87 7.74 μg/mL

4. The hydroxyl radical scavenging activity was also evaluated using the deoxyribose method. The acetone (successive) extract provided the highest potency (lowest IC_{50}) comparable to Trolox®. The spray-dried *P. emblica* was as active as EGCG, and were more potent than commercial *P. emblica* extract and the acetone (direct) extract. The ethanol (direct) extract exhibited the lowest hydroxyl radical scavenging activity as seen from its highest IC_{50} value. The ranking in an increasing order and grouping of the antioxidants with respect to their IC_{50} values against hydroxyl radical, are as follows:

acetone (successive) ≈ Trolox® < spray-dried *P. emblica* ≈ EGCG <

0.88 0.92 1.12 1.19 mg/mL

commercial *P. emblica* ≈ acetone (direct) < ethanol (successive) <

1.62 1.67 1.79 mg/mL

ethyl acetate < ethanol (direct)

2.88 2.97 mg/mL

Although the IC_{50} value of the ethyl acetate extract was significantly lower than the ethanol (direct) extract, it still provided the lowest hydroxyl radical inhibition profile in comparison with all test samples

5. It is interesting also to note that the antioxidant activities tended to reside within the more polar components of the fruit juice since the ethyl acetate extract (the least polar solvent used) gave comparatively low scavenging activity against both DPPH and hydroxyl radicals. However, this should be further proven by phytochemical analysis of the extract components.

6. Modification of the deoxyribose method by omitting l-ascorbic acid was carried out to investigate the pro-oxidant activity of *P. emblica* extracts compared to reference antioxidants at the concentration range of 1 to 3 mg/mL (except Trolox®) which was evaluated in the range of 0.05-2 mg/mL). All *P. emblica* extracts demonstrated pro-oxidant effects on deoxyribose degradation at lower concentration (different concentration for each sample) in the presence of metal ion. However, the oxidative stimulation by these extracts decreased at higher concentration. This could be due to the reducing property of their antioxidant components is predominant at the lower concentration but at higher concentrations the scavenging activity became dominant and the pro-oxidant effect was overcome.

7. Interestingly, Trolox® showed no pro-oxidant activity in the concentration range of 0.75 to 2 mg/mL. However, at the lower concentration of 0.05-0.5 mg/mL it exhibited some pro-oxidant activity.

8. Acetone (successive) extract was shown to have minimum pro-oxidant activity at 1 mg/mL when compared to other *P. emblica* extracts and EGCG. The ranking of the pro-oxidant activity at 1 mg/mL concentration, as judged from the absorbance increase (in folds) over their corresponding control (no test compound) is as follows:

acetone (successive) < ethanol (successive) ≈ spray-dried *P. emblica* <

1.03 1.27 1.28 fold

acetone (direct) < ethanol (direct) < commercial *P. emblica* < EGCG < ethyl acetate

1.37 1.41 1.57 1.66 1.97 fold

On the other hand, Trolox® at 1 mg/mL showed no pro-oxidant activity with the mean absorbance ratio relative to the control of less than 1 (= 0.94) whereas l-ascorbic acid, the reference pro-oxidant used in this study, demonstrated very high pro-oxidant activity. At the concentration of 0.1 mM (17.61 μg/mL), it showed the absorbance increase in the range of 2.15-2.66 folds (average = 2.40 folds).

9. Anti-collagenase activity was also tested using Enzchek® Gelatinase/ Collagenase assay kit. As judged from the IC_{50} values, 1,10-phenanthroline was shown to give the highest anti-collagenase activity (IC_{50} = 12.09 μg/mL). However, it is used for research purposes only. Both the ethanol extracts (direct and successive) apparently provided greater anti-collagenase activity than other *P. emblica* extracts, whereas the commercial *P. emblica* extract exhibited the lowest collagenase inhibitory activity. It also appeared that the anti-collagenase activity tended to reside within the more polar components of the fruit juice, with greater activity observed with acetone and ethanol than with ethyl acetate extract.

10. Regarding the anti-tyrosinase activity, all the *P. emblica* extracts provided some inhibitory activity but their effects were much smaller than licorice extract, the reference tyrosinase inhibitor used in this study. The ranking of their IC_{50} values, in an increasing order, is as follows:

licorice extract < ethyl acetate < acetone (direct) <

0.88 μg/mL 1.19 mg/mL 1.51 mg/mL

ethanol(direct)≈commercial *P.emblica*≈acetone(successive)≈ spray-dried *P.emblica* <

1.73 mg/mL 1.78 mg/mL 1.79 mg/mL 1.85 mg/mL

ethanol (successive) extract

2.35 mg/mL

11. It is also interesting to note that the anti-tyrosinase activity was greater in the ethyl acetate extract than in other extracts, implying that the tyrosinase inhibitory activity may reside in the less polar components of the *P. emblica* fruit juice. This was substantiated by the highest IC_{50} for the ethanol (successive) extract. It was contrary to the antioxidant activity, for which the ethyl acetate extract tended to be less active than when the more polar solvents (acetone and ethanol extracts) were used.

12. Preliminary stability of various *P. emblica* extracts was also evaluated for their antioxidant activity against DPPH radical. Spray-dried *P. emblica* exhibited only a slight decrease in activity (from 68.93% to 62.86% at 10 μg/mL and 92.45% to 89.16% at 20 μg/mL), whereas the other solvent extracts showed no decrease in activity at both concentrations after 9-month storage. Therefore, extraction with organic solvent could improve the stability of the aqueous-based extract like the spray-dried *P. emblica* powder. The stability profile of the spray-dried powder was also comparable to the commercial *P. emblica* extract, suggesting the possibility that the commercially available product may have been prepared by the same solvent-free process.

13. The antioxidant, anti-collagenase and anti-tyrosinase activities of various *P. emblica* extracts compared with the reference products can be summarized in the following table:

Table 23. Summary of antioxidant, anti-collagenase and anti-tyrosinase activities (expressed as average IC_{50} values) of the individual test samples.

Samples	Antioxidant			Anti-collagenase (μg/mL)	Anti-tyrosinase (mg/mL)
	DPPH radical (μg/mL)	$OH^{\cdot}$ Radical (mg/mL)	Pro-oxidant at 1mg/mL (fold)*		
Spray-dried *P. emblica*	6.29	1.12	1.28	121.13	1.85
Ethyl acetate	7.74	2.88	1.97	277.27	1.19
Acetone (successive)	5.00	0.88	1.03	45.51	1.79
Acetone (direct)	4.43	1.67	1.37	48.56	1.51
Ethanol (successive)	4.63	1.79	1.27	32.77	2.35
Ethanol (direct)	4.62	2.97	1.41	37.24	1.73
Commercial *P. emblica*	6.87	1.62	1.57	290.74	1.78
Trolox®	3.57	0.92	0.94**	-	-
l-ascorbic acid	2.37	NA	-	-	-
EGCG	1.52	1.19	1.66	-	-
1,10-phenanthroline	-	-	-	12.09	-
Licorice extract	-	-	-	-	0.88μg/mL

NA = not analyzed (l-ascorbic acid was used as one of the reagents in the deoxyribose method)

* pro-oxidant activity in folds higher than the ascorbic acid-free control

** no pro-oxidant activity at this concentration (1mg/mL)

14. In conclusion, the result from this study showed that solvent extractions of spray-dried *P. emblica* fruit juice have an effect on the antioxidant, anti-collagenase, and anti-tyrosinase activities. The ethyl acetate extract (low polarity) showed better anti-tyrosinase activity than the spray-dried *P. emblica*, and also gave higher activity than the acetone and ethanol extracts. However, it exhibited lower antioxidant and anti-collagenase activities than the spray-dried *P. emblica*, acetone and ethanol extracts. The direct and successive extraction processes are also important for the activity of *P. emblica* extracts. It was found that the successive extracts of acetone and ethanol tended to show greater overall antioxidant and anti-collagense activities than the direct extracts and, vice versa, demonstrated lower anti-tyrosinase activity than the

direct extracts. This result may be due to the co-extracted contaminating substances which may affect anti-tyrosianse activity, whereas antioxidant and collagenase activities need the successive extraction process in order to increase their activity. Regarding the anti-collagenase activity, both the ethanol (successive) and ethanol (direct) extract exhibited slightly greater collagenase inhibition than the acetone extracts. However, most of the acetone extracts provided higher potency on the combined antioxidant and anti-tyrosinase activities than the ethanol extracts when compared under the same extraction process. Moreover, the acetone extracts especially the acetone (successive) extract was safer than other *P. emblica* extracts and EGCG in the presence of metal ions with respect to the stimulation of hydroxyl radical regeneration. Therefore, the acetone (successive) extract should be selected to represent a refined grade of *P. emblica* extract that could provide high antioxidant and anti-collagenase plus some anti-tyrosinase activities. In addition, this extract may prove to be a safer antioxidant than commercial *P. emblica* extract in the presence of metal ion due to its minimal pro-oxidant activity.

15. Considering its multifunction, inexpensiveness, good long term stability and availability, *P. emblica* locally grown in Thailand has a very promising potential for use as an antioxidant, anti-collagenase and anti-tyrosinase agent in the cosmetic and other health-related industries.