CHAPTER V

DISCUSSION AND CONCLUSIONS

Hyperpigmentation is a psychosocial and cosmetically distressing problem caused by various forms of skin disorders, such as melasma, postinfammatory melanoderma, solar lentigo, allergic contact dermatitis, and irritant contact dermatitis. These skin diseases can be dependent on increased numbers of melanocytes or activity of melanogenic enzymes e.g. tyrosinase, resulting in an increased production of melanin pigments. Efforts have been devoted to screen recognized and putative depigmenting agents, including bleaching compounds which, in deed, are fairly ineffective on dermal accumulation of melanin. Physical therapies, such as lasers, have also been proposed and are currently under investigation. The ideal depigmenting compound should have a potent, rapid and selective bleaching effect on hyperactivated melanocytes without short- or long-term side-effects and lead to a permanent removal of undesired pigment, acting at one or more steps of the pigmentation process. [9, 10] The most popular depigmenting agent is hydroquinone (dihydroxybenzene; HQ), which is a hydroxyphenolic chemical. HQ seems to exert its effects mainly on melanocytes by targeting tyrosinase activity. However, because of the hazard of long-term treatments [8], the use of hydroquinone in cosmetics has been banned by the European Committee (24th Dir. 2000/6/EC) and formulations are available only by prescription of physicians and dermatologists. Instead of using HQ, natural tyrosinase inhibitors, such as herbal extracts have been subjected to current investigation. Recently, it has been reported that euphorbiaceous plants possess the tyrosinase inhibitory activity, which may be useful for developing novel compounds for the treatment of hypepigmentation [11, 12]

This thesis works were divided into four parts. The first part dealed with enzymatic reaction for the screening of tyrosinase inhibitory activity of the extracts from two euphorbiaceous plants namely *Mallotus spodocarpus* and *Excoecaria bicolor*, which are wildly distributed in Thailand, using mushroom tyrosinase assay. In the second part, the cytotoxic effects of both extracts were examined in melanocyte cell line using MTT cytotoxic/proliferation assay. In the third part, the effect of the extracts on RNA expression of tyrosinase, which is the rate-limiting step of melanogenesis pathway, and its transcription factor MITF is investigated by means of RT-PCR. In the last part, effect of the extracts on signaling cascades leading to the inhibition of tyrosinase expression was examined.

Results from the first part showed the extract from *Excoecaria bicolor* was more effective than that of *Mallotus spodocarpus* in inhibiting enzymatic activity of tyrosinase, suggesting that *Excoecaria bicolor* extract may contain a larger proportion of chemical structures that could inhibit tyrosinase activity such as compounds bearing hydroxyphenolic structure similar to that of HQ. The finger print HPLC analysis (see appendix E) confirmed the presence of a large fraction of gallic acid, which has a hydroxyphenolic structure similar to that of HQ [78] in *Excoecaria bicolor* extract. Moreover, our unpublished data indicated that purified gallic acid inhibited mushroom tyrosinase activity.

In this part, MTT cytotoxic/proliferation assay was employed to examine the cytotoxic effect of the extract. In this assay, viable melanocytes converted the MTT substrate that put in and change with the dehydrogenase enzyme to the product that can measure with microplate reader. The result has shown that *Mallotus spodocarpus* extract is more cytotoxic to melanocytes than that of *Excoecaria bicolor*. The cytotoxic effect of *Mallotus spodocarpus* extract could due to the presence of a large amount of saponin in the extract, as indicated by finger print analysis. High

concentration of saponin can disrupt plasma membrane integrity leading to cell death. [79] This observation was also in agreement with the absence of saponin in *Excoecaria bicolor* extract and relatively low cytotoxicity to melanocytes.

Alternatively, the process of depigmentation can be achieved by regulating at the level of gene expression of tyrosinase and its transcription factor MITF. Here, the result showed for the first time that the crude extracts from *Mallotus spodocarpus* and *Excoecaria bicolor* could reduce the expression of these two transcripts. The reduction of MITF RNA by the extracts was consistent with the down-regulaion of tyrosinase. However, basal expression level of tyrosinase RNA in melanocytes was relatively high, compared to that of MITF. Then, I further explored other possible mechanism, including signal transduction involved in melanin synthesis that could control the expression of tyrosinase in melanocyte. Because MITF is among other substrates of phosphorylated ERK leading to MITF degradation [5], I therefore examined whether *Excoecaria bicolor* extract could affect protein expression of phosphorylated ERK. The results showed that *Excoecaria bicolor* extract increased phosphorylated ERK in a does-dependent manner; suggest that regulatory effect of the extract may involve the activation of ERK leading to MITF degradation, subsequently a downregulation of tyrosinase RNA.

In summary, the findings indicate that the crude extract from *Excoecaria bicolor* possesses anti-tyrosinase activity and its mechanism of action may involve a down-regulation of tyrosinase and MITF RNA, as well as ERK-mediated MITF protein degradation. Therefore, this work scientifically proves that the crude extract from *Excoecaria bicolor* may be useful for developing novel compounds for the treatment of melasma and other hyperpigmentation.