# CHAPTER 4

# CONCLUSION

The results from this study can be concluded as the followings:

1. The extracts from the three medicinal flowers including the flower Bullet Wood *(Mimusops elengi*; ME, Sapotaceae), Cork Tree (*Millingtonia hortensis*; MH, Bignoniaceae) and Cape Gardenia (*Gardenia jasminoides*; GJ, Rubiaceae) by the two non-heated processes supereritical carbon dioxide fluid extraction ($scCO_2$) and hexane maceration were prepared.

2. The flower extracts from MH, ME and GJ by $scCO_2$ with 33 % (w/v) of 95% (v/v) ethanol showed the highest percentage yields at 14.40 ± 1.25, 9.61 ± 1.25 and 8.78± 1.27, respectively.

3. The extracts were identified by Gas chromatography-mass spectrometry (GC-MS) and High Pressure Chromatography (HPLC). The results of their chromatograms and volatile compositions of the hexane extracts were phenyl ethyl alcohol 15.09 %, cinnamyl alcohol 6.76 %, and cinnamic aldehyde 3 % for ME, pentadecane 14.56 %, linalool 13.73 %, n-dodecane 8.46 % for MH and alpha farnesene 15.14 %, tetratriacontane 8.46% and benzyl salicylate 8.56 % for GJ.

4. The chemical constituents of the extracts were γ-linoleic 1.06% w/w and oleic acid 0.54 % w/w in MH, while γ-linoleic 0.07 % w/w in ME and γ-linoleic 0.02 % w/w, oleic acid 0.14 % w/w in GJ. The phytochemical test revealed that ME, MH and GJ contained xanthone and flavone, while GJ and MH also contained carotenoid.

5. The biological activities of the extracts by the supercritical carbon dioxide fluid ($scCO_2$) with the co-solvent (ethanol) at 4, 20, 33 and 50% (w/v) were compared with the extracts prepared by hexane maceration. All extracts by $scCO_2$ gave higher free radical scavenging and skin fibroblast proliferation activities than those by the hexane maceration.

6. The $scCO_2$ extracts of ME, MH, and GJ prepared with 20, 33 and 33% (w/v) ethanol as a co-solvent which showed the high percentage yields and free radical scavenging activity, were selected to investigate for MMP-2 inhibition.

7. The extract of GJ by $scCO_2$ with 33% (w/v) ethanol as a co-solvent demonstrated the highest MMP-2 inhibition activity (43.85 ± 9.61%), high percentage yield (8.78 ± 1.27%w/w), high free radical scavenging activity ($SC_{50}$ at 5.14 ± 0.32 mg/ml) and low toxicity on human skin fibroblast (% cell growth of 68.48 ± 5.95 %).

8. The ME, MH and GJ extracts prepared by $scCO_2$ were loaded in niosomes [composed of Tween 61/cholesterol at 1:1 molar ratio] by the chloroform film method with sonication. The maximum loading concentration of all flower extracts in niosomes was 1% w/v.

9. The niosomes both non-loaded and loaded with the flower extracts showed high physical stability with no change of color and precipitation when kept at various temperatures ($4\pm2$, $27\pm2$ and $45\pm2^{O}C$) for 3 months.

10. The ME extract loaded and non-loaded in niosomes exhibited the free radical scavenging ($SC_{50}$ ), metal chelating ($MC_{50}$) and lipid peroxidation inhibition ($IPC_{50}$) at $9.80\pm0.85$, $32.15\pm0.34$ and $16.69\pm7.35$ mg/ml; $6.82\pm0.73$, $0.46\pm0.09$ and

0.45±0.02 mg/ml, respectively, while the metal chelating activity was not observed in the MH and GJ extracts.

11. When loaded in niosomes, the cytotoxicity of all flower extracts on human skin fibroblast determined by the sulforhodamine B assay was decreased of 1.40-1.70 times.

12. The ME extract loaded in niosomes exhibited higher active MMP-2 inhibition than the non-loaded extracts of 3.4 times.

13. All flower extracts both loaded and non-loaded in niosomes gave no irritation on the rabits'skin by the close patch test.

14. For the cost estimation, it indicated that ME niosomes was 292.73 Bath/100 ml, MH niosomes was 250.24 Bath/100ml and GJ niosomes was 295.25 Bath/100ml.

15. The niosomes loaded with all flower extracts especially the ME extract can be applied for the further development of cosmetic products.

For the suggestion, the further *in vivo* evaluation in human volunteers of the selected niosomes loaded with the selected flower extract incorporated in the suitable cosmetic product should be performed in order to evaluate its potential for cosmetic uses. In addition, because of the current objection of using animal test both in EU and AEC, an alternative test for skin irritation using *in vitro* or clinical testing in human should be used for the substitution of the rabbits' skin close patch test.