CHAPTER V

CONCLUSION

For this study, green tea from three different Asian sources namely; Japanese green tea (JGT), Myanmar green tea (MGT) and Thai green tea (TGT), which are available on the market with good reputation, were selected randomly. Then the investigations among green tea extracts *in vitro* were carried out and their contents and activities were compared in terms of total polyphenol content, antioxidant activity and cytotoxicity.

Green tea extracts were obtained in the form of light-brown solid matter after extraction. The quantitative analysis of green tea polyphenols from each extracts were carried out by using high performance liquid chromatography (HPLC) assay. On the basis of the quantitative analysis percent yield of the total polyphenol content in each of green tea extracts was observed as follows; JGT; 58%, MGT; 55% and TGT; 54% (w/w) of the dry solid weight.

From this investigation, it was found that all three green tea extracts from different sources were rich in total polyphenol contents. Epicatechine derivatives present in JGT ranked in the order of EGCG>EGC>ECG>EC. But the presence of epicatechine derivatives in MGT and TGT were in the same order as follow; EGCG> ECG>EGC>EC. Nevertheless, the major constituent present in all green tea extracts was found to be EGCG with JGT having the highest total polyphenol contents and also the highest EGCG content when compared with other green tea extracts; MGT and TGT. However, no significant difference was observed in comparison with total polyphenol content among green tea extracts, $p>0.05$.

Another strategy in this study was to evaluate the antioxidant properties of green tea polyphenols. Generally, because of the complex nature of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by one single method.

In the meantime, many strategies have been developed and well established to assess the antioxidant activities. In the present study, the antioxidant activity of green tea extracts was evaluated *in vitro* by using two methods; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay and total antioxidant status assay.

In DPPH assay, green tea extracts were screened for their radical scavenging activity on stable DPPH radical. From this investigation, the concentration required for 50% reduction on DPPH radical (EC_{50}) was detected at 2.80, 2.94 and 3.07 μg/ml in the case of JGT, MGT and TGT respectively. It was concluded that all green tea extracts from three different sources showed a dose-dependent free radical scavenging effects on DPPH radical and the effectiveness in inhibition at 50% level on DPPH radical in the order of JGT>MGT>TGT. Statistical difference among the three green tea extracts on DPPH radical at EC_{50} was observed, $p<0.05$.

Moreover, in comparison with selected antioxidant BHT, EC_{50} of green tea extracts were five times comparatively lower and statistically significant, $p<0.05$. This study demonstrated that all three green tea extracts have low EC_{50} with with strong radical scavenging effects on DPPH radical in comparison with BHT.

In order to verify the results from DPPH assay, the radical scavenging activity of green tea extracts were measured in terms of total antioxidant status assay by using Randox kit method with ABTS radical cation ($ABTS^{\cdot+}$). Total antioxidant activity of three green tea extracts were expressed in terms of mmol/l with Trolox (water-soluble vitamin E analog) used as a standard. In the present study, the total antioxidant activity of all green tea extracts were 0.95, 0.86 and 0.83 mmol/l Trolox equivalent for JGT, MGT and TGT respectively. In going through the comparative study of the total antioxidant activity, no significant difference was observed among three green tea extracts.

In vitro, the cytotoxicity of green tea extracts were evaluated with melanoma cell line (A375) by using 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. The tendency of the test sample to inhibit the melanoma cell

line was observed with wide range of concentration and the dose response curve was plotted. The effective concentration of each extract at 50% inhibition (EC_{50}) level on melanoma cell line was calculated from the curve and their results were compared. EC_{50} was obtained at 173 μg/ml for JGT, 198 μg/ml for MGT and 206 μg/ml for TGT. It was concluded that all green tea extracts have cytotoxic effects on melanoma cell line and significant difference was observed in the comparison of the cytotoxicity among the three green tea extracts at EC_{50} in the order of JGT>MGT> TGT. JGT showed the strongest cytotoxic effect on melanoma cell line at EC_{50} level followed by MGT and TGT.

From overall *in vitro* investigations in this study, three green tea extracts ranked in the order of JGT followed by MGT and TGT, in terms of the total polyphenol content, the radical scavenging activity, the total antioxidant activity and the cytotoxicity. Strongest activity of JGT among green tea extracts seemed to be due to the presence of highest polyphenol contents and its major constituent EGCG, which is claimed to have high antioxidant and anticarcinogenic activity as well.

In comparison among green tea extracts, significant difference was observed in the study of the radical scavenging activity and the cytotoxicity but in the case of the total polyphenol content and total antioxidant activity not much significant difference was experienced. In summarizing all these findings, it was concluded that all green tea extracts from three different Asian countries have been found to have antioxidant activities contributing to the anticarcinogenic activities.

In fact, as all the experiments are *in vitro* study only, these effects may or may not reflect the same way *in vivo*. The mechanism and effects of green tea polyphenols *in vivo* are yet to be fully understood and much remains to be developed. From this point, more research will be required *in vivo* study of green tea, from the basic level up to the molecular level. Beside, the choice of tea sample with its origin, method of productions and quality are points to be considered in future studies. Moreover, there is still room for further extensive study in improving

the extraction methods with a view to having high yield of green tea polyphenols to be studied with wide varieties of brands from each source.

Nevertheless, findings from the above studies may however provide the ground work for alternative treatment of cancer with natural products, which can overcome the toxic and unpleasant treatments experienced with current chemotherapy and radiation treatments. Hopefully, all the above findings will provide the basic information for future studies to formulate skin care products with green tea extracts.