

CHAPTER 5

CONCLUSION

Due to the increasing demand and interest in the consumption of medicinal plants as an alternative therapy, it is thus important to carry on some investigation to support the therapeutic claims. Besides that, investigations of plants also ensure that the plants are safe for human consumption.

Traditionally *P. oleracea* has been used for the treatment of cancer related diseases by locals. The results from the current studies thus provide scientific validation on the use of whole plant of *P. oleracea*.

Bioassay-guided fractionation was the experimental approach in the present study. The crude methanol and fractionated extracts of *P. oleracea* were initially prepared and then subjected to bioassay-guided investigation. The bioactive fractions of *P. oleracea* were then identified through these investigations.

In comparison to other *P. oleracea* extracts, the ethyl acetate fraction showed the highest antioxidant activity when determined using the scavenging effect on DPPH radicals and reducing power assay. In the β -carotene bleaching assay, it was the water fraction that showed the highest antioxidant activity. The highest antioxidant activity shown by the ethyl acetate fraction in the DPPH assay indicated that the compounds with the strongest radical scavenging activity in *P. oleracea* were of medium polarity. Whilst, those responsible for the activity in the β -carotene bleaching assay were polar in nature.

The extracts of *P. oleracea* were then investigated for their cytotoxic effects against selected human cell lines namely the hormone-dependent breast carcinoma cell

line (MCF7), human cervical carcinoma cell line (Ca Ski), human colon carcinoma cell line (HT-29), human colon carcinoma cell line (HCT 116), human lung carcinoma cell line (A549) and non-cancer human fibroblast cell line (MRC5) using an *in vitro* neutral red cytotoxicity assay.

Only the ethyl acetate fraction of *P. oleracea* gave the highest inhibition and stimulation values against various cancer cell lines when compared to other extracts. The ethyl acetate fraction was selectively toxic against HT-29 cells exhibiting a remarkably IC_{50} value of $8.00 \pm 0.58 \mu\text{g/ml}$. The active compounds in the ethyl acetate fraction were able to kill HT-29 cells but exert no damage to the normal cells MRC5 ($IC_{50} > 100.00 \mu\text{g/ml}$). The ethyl acetate fraction also showed moderate cytotoxic profile against HCT 116 cells with IC_{50} value of $32.00 \pm 3.06 \mu\text{g/ml}$. The hexane fraction of *P. oleracea* also showed weak cytotoxic profile against HT-29 with IC_{50} value of $39.00 \pm 3.06 \mu\text{g/ml}$. The methanol extract, hexane and water fractions did not demonstrate cytotoxic activity against MCF7, Ca Ski, A549, HT-29, HCT 116 and MRC5 ($IC_{50} > 100.00 \mu\text{g/ml}$).

Analysis of the hexane fraction of *P. oleracea* by GC-MS showed that it contained methyl palmitate (11.10 %), methyl oleate (2.88 %), methyl linoleate (4.07 %), methyl linolenate (8.70 %), phytol (41.55 %), palmitic acid (7.86 %) and squalene (19.81%).

Mixture (I) and mixture (II) isolated from the active ethyl acetate fraction did not exhibit cytotoxic effect on HT-29 cells. Whilst, mixture (III) and mixture (IV) exhibited weak cytotoxic effect on HT-29 cells. The cytotoxic activity of the isolated mixtures alone on HT-29 cell line was found to be lower than that shown by the ethyl acetate fraction from which the mixtures were obtained. The mixture of compounds present in the total ethyl acetate fraction was responsible for the strong cytotoxic activity. The

interaction between the different biological active components in the extract may be responsible for their total effects. The different compounds can modulate unrelated signaling pathway and therefore result in synergistic effects. The ethyl acetate fraction of *P. oleracea* contained a combination of active constituents which interacted within themselves and to enhance (synergize) the therapeutic effect. Semi-purification process ended up in loss of biological activity indicating that synergistic activities occurred between the various components. Some components present in the ethyl acetate fraction were positively identified as friedelin, β – sitosterol, campesterol, 3-buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl) and 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one through GC-MS and LC-MS/MS analysis. There are many other components that were not identified.

The cytotoxic activity of ethyl acetate fraction was not as effective as doxorubicin. However, it has low toxicity against normal MRC5 cell line. The ethyl acetate fraction may be used in combination with established cytotoxic therapeutic drugs to reduce the side effect of the synthetic drugs. It is suggested that *in vivo* studies involving experimental mice be pursued to support this belief and to ensure the safe consumption of the plants.

In conclusion, the result obtained from the present study support the common belief that ethnopharmacological selection of *P. oleracea* is a useful criterion in drug discovery.