CHAPTER 1

INTRODUCTION

Naturally derived products are the basis of most early medicines. Natural products are chemical compounds or substances derived from living organisms and classified as secondary metabolites. Many pharmaceutical agents have been discovered through screening of natural products from plants, insects, marine organisms, microbes and animals. Natural products have a great variety of structural diversity which can provide valuable sources of novel compounds for the discovery of therapeutic targets.

Around 250,000 plant species are found all over the world and most of them are located in the tropical rainforests. Malaysia has about 15,000 species of flowering plants of which about 10 % are said to be medicinal (Faridah Hanum *et. al*. 2001a; Faridah Hanum *et. al*. 2001b). The huge diversity of chemical structures from natural products makes them excellent candidates for any bioactivity screening programme.

Enthopharmacological data provides useful information on the traditional uses of plants. Thus selection of plants based on enthopharmacological data is a good criterion for study. Ethnomedical data provides substantially increased chance of finding active plants relative to random approach (Chapuis *et al*., 1988). Primary metabolites and secondary metabolites are found in plants. Primary metabolites are needed to perform metabolic roles. Meanwhile, secondary metabolites do not directly participate in the growth and development. Studies have shown that these secondary metabolites possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, antiviral or anti-malarial activities (Halliwell, 1994; Mitscher *et al*., 1996; Owen *et al*., 2000; Sala *et al*., 2002).

Over the period 1957 - 1981, around 20,000 plant species from Latin America and Asia were screened for anti-tumor activity by the National Cancer Institute (NCI),

but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991). Between the years 1981 and 2002, 1031 new chemical entities (NCEs) have been discovered. Nowadays, about 25 % of drugs prescribed worldwide are of plant origin. The interest in drugs of plant origin is increasing because synthetic drugs may result in side effects and other problems. Folk medicine and ecological awareness suggest that the natural products are fewer side effects.

Cancer is a type of disease characterized by abnormal cells which divide without control. Cancer cells do not undergo programmatic death (apoptosis) when they should. These cells will grow and divide continuously and lead to a mass of cells. The mass of cells may form a mass of tissue known as tumor. Cancer cells are able to spread to the whole body through blood and lymph systems. A large number of natural compounds from vegetables and herbs were found to exert chemopreventive properties against carcinogenesis. For example, a natural product namely silvestrol isolated from *Agaila sylvestre* exhibited cytotoxicity against lung and breast cancer cell lines (Cragg and Newman, 2005). *Rhizoma zedoariae* produces a compound called lemene. This compound able to inhibit antitumor activity in human and murine tumor cells *in vitro* and *in vivo* (Zheng *et al.*, 1997). Vincristine isolated from *Catharanthus roseus* is used to treat leukaemia, malignant lymphoma and lung cancer (Finn and Desmond, 2001). Whilst, taxol isolated from *Taxus brevifolia* is used in the treatment of ovarian cancer (Finn and Desmond, 2001).

Free radicals have been implicated in many diseases including cancer. Free radicals are atoms or atomic groups that contained one or more unpaired electron in the outermost orbit. They are highly reactive. Free radicals will indiscriminately pick up electron(s) from the nearest stable molecules. Free radicals will react with molecules such as proteins, lipid, carbohydrate and DNA. Superoxide, hydroxyl, hydroperoxyl, alkoxyl, peroxyl and nitric oxide radicals are examples of free radicals. Oxidative stress

occurs when there is an imbalance between productions of reactive oxygen species (exogenous sources or endogenous sources) against antioxidant protection mechanism (enzymatic and non-enzymatic) in an organism. Oxidative stress will lead to cell or tissue injury and finally causing many chronic and degenerative diseases such as atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Diaz *et al*., 1997; Lang and Lozano, 1998; Halliwell, 2000; Metodiewa and Koska, 2000; Young and Woodside, 2001; Heinecke, 2003; Leibovitz and Siegel, 1980).

Antioxidants have the ability to donate their electron(s) to the free radicals. The chain reaction of oxidation is stopped when an antioxidant donates electron(s) to the free radicals to neutralize the free radicals. The commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), propylgallate (PG), *tert*-butylhydroquinone (TBHQ) and butylatedhydroxytoluene (BHT) (Sherwin, 1990). BHA and BHT were found to cause liver damage and carcinogenesis in experimental animals (Grice, 1986; Wichi, 1988). Thus it is important to find an alternative source of antioxidant from plants which is safer than the synthetic antioxidants.

Portulaca oleracea is an annual, succulent herb in the family Portulacaceae. This plant is given the term 'Global Panacea' by Dweck (2001). *Portulaca oleracea* (*P. oleracea*) are commonly known as Purslane, Pursley, Pusley, or Wild Portulaca. In Malaysia, this plant is known as '*Gelang Pasir'*. *P. oleracea* is found in various parts of temperate as well as tropical regions. The plant has radial grow pattern. The stems are glabrous, branching and succulent and flushed red or purple in colour. The leaves are succulent and the flowers are bright yellow. It is eaten as salad and vegetable all around the world. This plant is edible with a slightly acidic and salty taste similar to spinach. The leaves of *P. oleracea* are used for poulticing tumours, bad wounds and ulcers, and oedematous swellings. It is also used as antiphlogistic (Reid, 1993),

diarrhoea, haemorrhoids, enterorrhagia (Keys, 1976). It is also described as antidote, refrigerant and antidysenteric (Reid, 1993). *P. oleracea* exhibits a wide range of pharmacological effects including antibacterial (Zhang *et al*., 2002), analgesic, antiinflammatory (Chan *et al*., 2000), skeletal muscle- relaxant (Parry *et al*., 1993) and wound healing (Rashed *et al*., 2003) activities, bronchodilatory effect (Malek *et al*., 2004) and anti-fatigue activities (Yue *et al*., 2005; Dong *et al*., 2005; Ling 2004). It is reported that extracts of *P. oleracea* has inhibitory effect on lipopolysaccharide (LPS) and interferon- ã (IFN- ã) induced NO production (Abas *et al*., 2006). Although *P. oleracea* is reported traditionally used in medicinal preparation, there is limited recorded data on its cytotoxic activity against various cell lines.

Hence, it is interesting to investigate the antioxidant and cytotoxic activity of *P. oleracea* in the present study. The experimental approach is based on bioassay-guided fractionation.

The main objective of this study is to investigate the antioxidant and cytotoxic activities of *P. oleracea*.

The specific objectives are as the following:

1. to determine the antioxidant properties of *P. oleracea* fractions and extract using DPPH free radical scavenging assay, ß-carotene bleaching assay and reducing power assay.
2. to screen for the cytotoxic activity of *P. oleracea* fractions and extract by an *in vitro* growth inhibition assay system (Neutral Red assay) against human cancer and non-cancer cell lines namely an estrogen positive (ER^+) mammary adenocarcinoma cells (MCF7); epidermal carcinoma cervical cell line (Ca SKi); colon cancer cell line (HT-29); colon cancer cell line (HCT 116); lung cancer

cell line (A549); nasopahryngeal cancer cell line (KB) and the normal lung fibroblasts cell line (MRC5).

3. to determine the chemical compounds in the hexane fraction of *P. oleracea*.
4. to evaluate the cytotoxic activity of the isolated mixtures from the active ethyl acetate fraction through bioassay guided techniques.

The outline of general procedures of this research project is as shown in Figure 1.1.

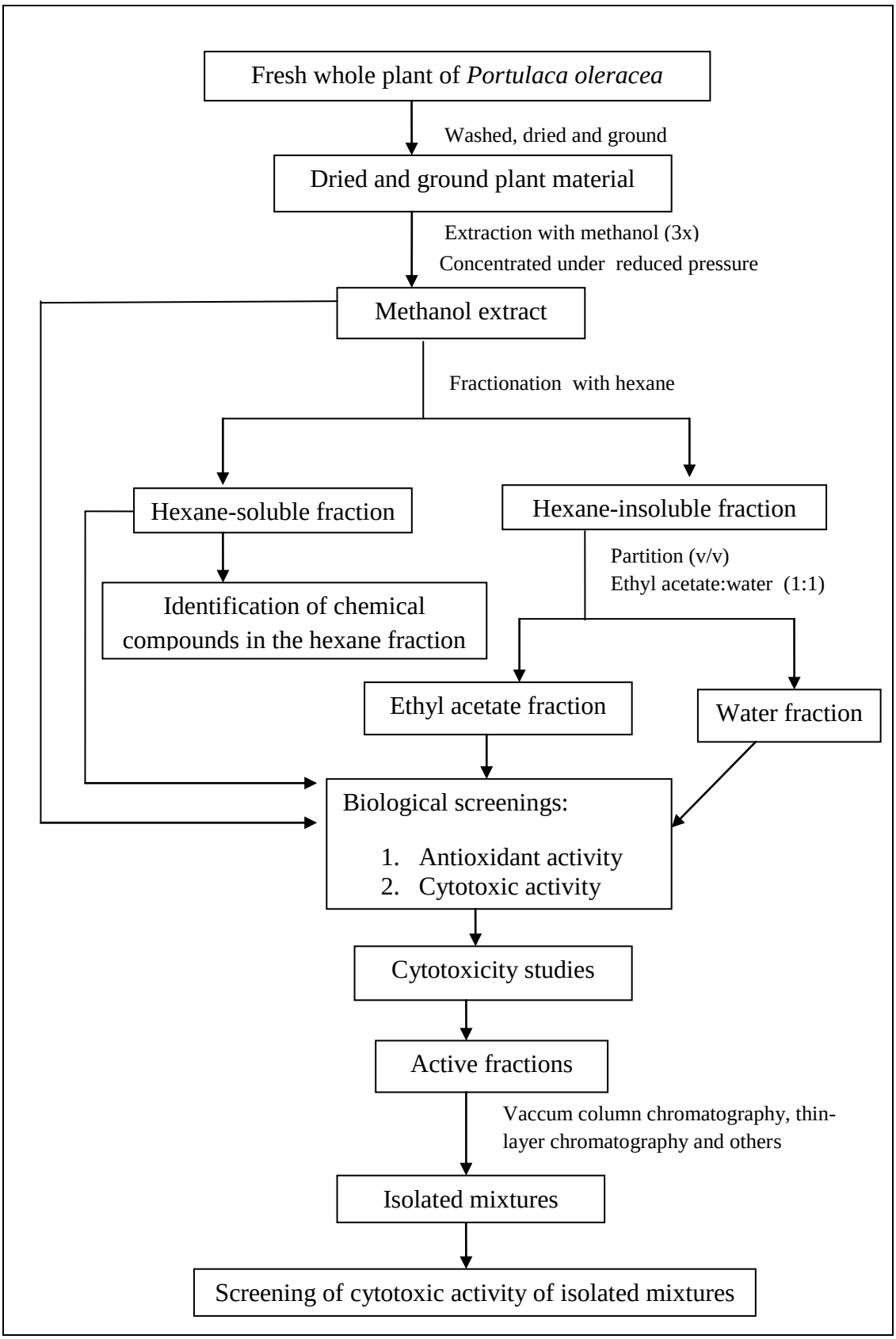

Figure 1.1: Outline of general procedures