

## CHAPTER 1

### INTRODUCTION

It is always an interesting target for scientists over decades to study on natural products especially on plants. Historically, plants (fruits, vegetables, medicinal herbs) have provided a good source of wide variety of compounds, such as phenolic compounds, nitrogen containing compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities, for examples, antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, anti-tumor anti-bacterial, or anti-viral activities. Traditional herbal medicines have been widely used for thousands of years in many oriental countries such as China, Thailand and India. Herbal plants have recently become the main object of chemists, biochemists, and pharmacists (Ly *et al.*, 2002; Juntachote *et al.*, 2006). There is a growing shift of people choosing herbal cures over conventional drugs due to the perception that herbals are safer and have reduced side effect (Ramlan, 2003). Besides that, clinical tests using herbal medicine have shown no adverse effect on renal, hepatic, and bone marrow function (Yuan and Lin, 2000).

Other than herbal medicine properties, natural compounds which are produced by plants, fungi, bacteria, protozoans, insects and animals provide us with diverse chemical structures. This gives us an opportunity to discover bioactive chemical structures to treat disease such as cancer. According to Lam (2007) and Newman *et al.* (2003), approximately 60 % of anti-cancer agents were derived from plant natural products and a further 20 % were natural products mimics or synthetic compounds derived from natural products. Hence, natural compounds from plants not only serve as

drugs but they also provide a rich source of novel structures that can be developed into novel anti-cancer agents. Most of the active compounds can be found in the wood, bark, stem, leaf, fruit, root, flower and seeds of many plants (Kris-Etherton *et al.*, 2002). For example, vincristine from Madagascar periwinkle and taxol from the bark of the Pacific Yew tree.

Cancer is one of the leading causes of death in both developed and developing countries and continues to be a major public health problem in many parts of world (Jemal *et al.*, 2010). Epidemiologic studies have shown that cancer is caused by several factors such as exposure to environmental carcinogenic agents, occupational environment, lifestyle, nutritional habit and infectious agents (Murthy and Mathew, 2004). Many studies and experiments have been carried out on natural sources from plants, marine and microorganisms to identify components which may have anti-cancer properties and to carry out *in vivo* experiments involving animals to ensure the safety. Resveratrol, quercetin and lycopene are examples of compounds utilized in cancer chemoprevention (Clinton, 1998). Existing therapies such as chemotherapy are not very specific for cancer cells and cause damage to normal tissue such as bone marrow, gut lining and hair follicles resulting in infection, diarrhea, vomiting and hair loss (Gore and Russell, 2003). This leads to the search for more effective and specific treatment against cancer.

The systemic screening of plant for cytotoxic activity started with National Cancer Institute (NCI), USA in 1960. NCI had screened over 114, 000 plant extracts and yielded taxol and camptothecin as anti-cancer agents. Another notable large scale screening programmes include the work done by the Council for Scientific and Industrial Research (CSIR) in South Africa that identified anti-cancer agents from plant natural products between 1998 - 2006 (Fouche *et al.*, 2008).

On this planet, the number of higher plant species (angiosperms and gymnosperms) are estimated at 250, 000 with lower level at 215, 000 and upper level as high as 50, 000 species. However, it has been estimated that only 6 % of tropical plants have been screened for biological activity and 15 % have been evaluated phytochemically (Fabricant and Farnsworth, 2001). Malaysia is listed among the 12<sup>th</sup> most biodiverse countries in the world with over 15, 000 flowering plants of which 3, 000 species are medicinal plants. Among the medicinal plants, only about 50 are used commercially and even less are being researched scientifically for their medicinal properties (Ramlan, 2003). *Alpinia*, from the Zingiberaceae family, is considered a large and taxonomically complex genus with 230 species widely distributed throughout tropical and subtropical regions of Asia. Many *Alpinia* plants are medicinal herbs and have been reported to have antioxidant, anti-inflammatory, anti-cancer, immunostimulating, hepatoprotective and anti-nociceptive activities (Matsuda *et al.*, 2003).

*Alpinia scabra*, locally known as 'Lengkuas raya', is an aromatic, perennial and rhizomatous herb. It is a wild species which grows largely on mountains at moderate elevations in Peninsular Malaysia, but it can also survive in lowlands like in the states of Terengganu and Northern Johor (Ibrahim *et al.*, 2010). There is only one published scientific report on *A. scabra* (Ibrahim *et al.*, 2010) regarding the cytotoxic activity of leaf and rhizome extracts of *A. scabra*. However, the previous report did not carry out detailed study to evaluate the cytotoxic and apoptotic effects of *A. scabra* on the human cancer cell lines. The experimental approach in the present study is based on bioassay-guided fractionation. In this endeavour, the crude methanol and fractionated extracts of *A. scabra* leaves, rhizoms, roots and pseudo stems were firstly subjected to cytotoxicity assay in order to identify the cytotoxic extracts. After identifying the cytotoxic extracts, the cytotoxic active extracts were subjected to isolation and purification procedures to

obtain chemical constituents present in the extracts and the cytotoxic fractions were further tested for their cytotoxic effect and subjected to apoptosis detection. The general procedures in the present study are outlined in Figure 1.1.

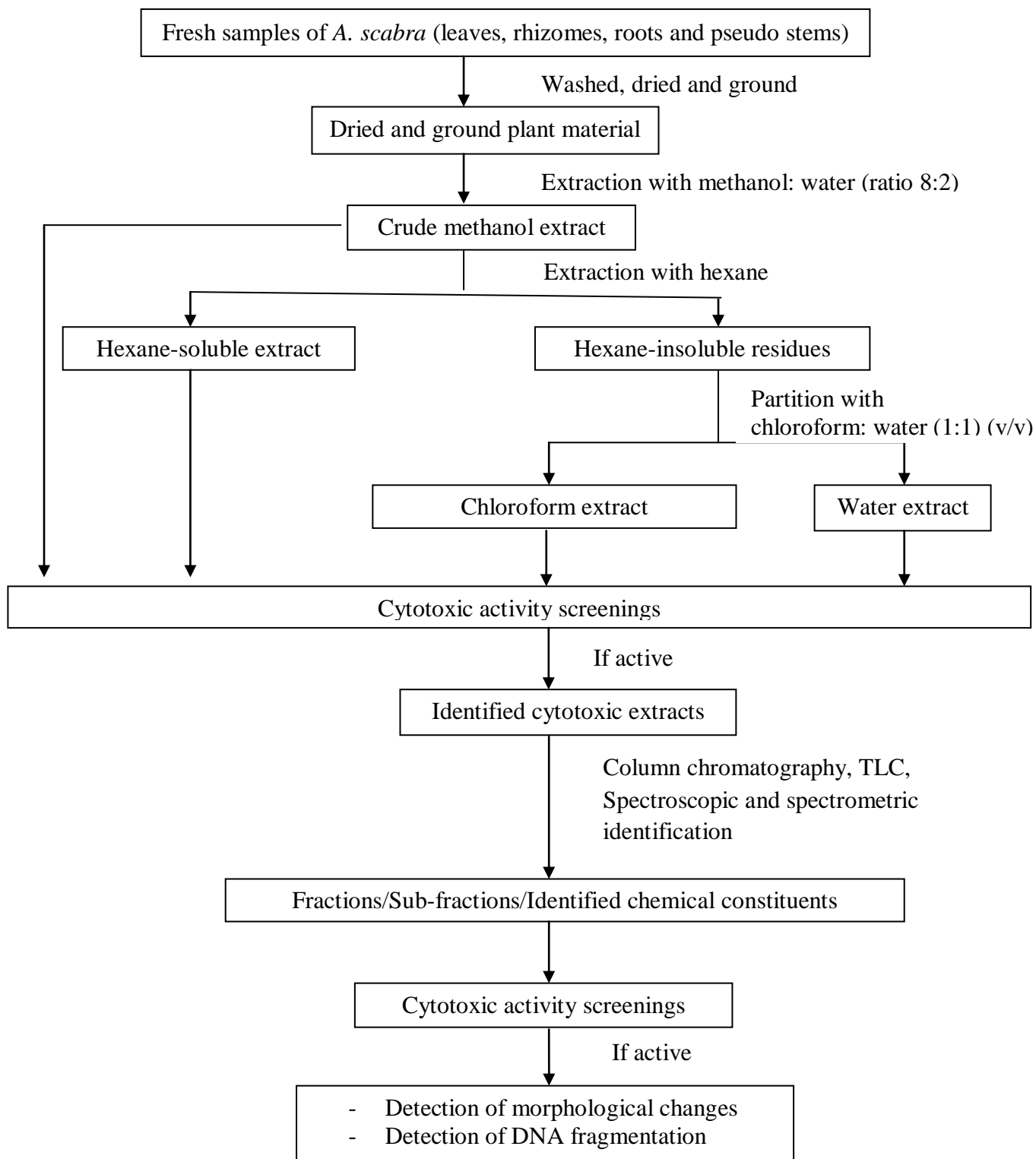
### **Objectives of study:**

The objectives of the present study were as follows:

- a) To evaluate the cytotoxic properties of the extracts of *A. scabra*
- b) To identify the bioactive fractions through bioassay guided fractionation technique
- c) To evaluate the cytotoxic activity of the bioactive fractions using *in vitro* cytotoxicity assay
- d) To detect the occurrence of apoptosis in treated cancer cells, namely, hormone-dependent breast carcinoma cell line (MCF7) and ovarian cancer cell line (SKOV-3).

### **Hypothesis of study:**

*A. scabra* may have the potential to be developed as cancer chemo preventive agent.



**Figure 1.1: Outline of general procedures**