



CHAPTER I

INTRODUCTION

Background and Rationale

Cancer is still one of public health problems in the world as well as in Thailand. Cancer cells are arisen from multistep processes of the progressive transformation of normal cells into highly malignant cells. It is generally known that the main characteristics of cancer cells are dysregulated proliferation, evasion of apoptosis, and ability to metastasis. Killing, removing, blocking proliferation and inducing apoptosis of cancer cells are strategies of cancer treatment. The clinical modalities to treat cancer are surgery for removing solid tumor, radiation for killing cancerous cells, and anticancer agents for killing, inhibiting proliferation and inducing apoptosis. Several types of anticancer agents have been used to treat various types of cancer. Cytotoxic agents are the main type of anticancer agents commonly used in combination to treat various kinds of cancer. Several problems from using cytotoxic agents to treat cancer, including severe side effects and drug resistance, lead to the continuous identification, investigation and development of novel anticancer agents.

Natural products have been recognized as a source for developing new drugs for a long time. Several natural products and natural-derived products are clinically used in cancer treatment. They are antibiotic anticancer drugs from microorganisms and several plant-derived anticancer agents. The most well known anticancer agents from plants are vinca alkaloids, vincristine and vinblastine, from the periwinkle plant *Catharanthus roseus*. Other plant-derived anticancer agents are etoposide which is epipodophyllotoxin derivatives from the mandrake plant and *Podophyllum peltatum* and the wild chervil *Podophyllum emodi*, two taxanes (paclitaxel and doxorubicin) from the bark of the yew tree *Taxus brevifolia* or the leaves of *Taxus baccata*, and camptothecin analogs (topotecan, irinotecan) derived from *Camptotheca acuminata*, *Ophiorrhiza*

pumila and *Mapia foetida* [1, 2]. Some natural product derived anticancer drugs have been approved and many of them are currently in several stages of drug development process [3].

Plants in the Rutaceae family contain several compounds that have a plethora of biological activities including antibacterial, antifungal, anti-tuberculosis and antitumor [4-6]. Carbazole alkaloids and coumarins are compounds of plants in this family that have been report in apoptotic induction activity and their effects on cell cycle disturbances [7-8]. Plants in the genus *Micromelum* of the Rutaceae are known to have carbazole alkaloids as parts of their active compounds which can induce apoptosis of U937 leukemiic cells [9]. Mahanine is the well known carbazole alkaloid which is a major constituent in *M. minutum*. It exhibits many pharmacological activities including anti-mutagenic, antimicrobial, anti-oxidants, anti-inflammatory and anti-tumor activities [10]. It has also been identified that the dichloromethane extract of the stem bark of *M. hirsutum* contains six carbazole alkaloids and one lactone derivative of oleic acid. There are one new carbazoles, micromeline and five known carbazoles, lansine, 3-methylcarbazole, methyl carbazole-3-carboxylate, 3-formylcarbazole, and 3-formyl-6-methoxycarbazole. The lactone derivative of oleic acid is micromolide. This extract has been demonstrated to exhibit anti-tuberculosis activity [5]. Although, carbazole alkaloids and coumarins derived from plants in genus *Micromelum* known to have anti-tumor activity, all of these compounds from *M. hirsutum* have no reported on antitumor activity. So, this study intended to investigate the cytotoxicity of compounds from the leaves and the branches of *M. hirsutum* on human B-lymphoma cells.

Objectives

1. To screen the cytotoxicity of solvent extracts from the branches and the leaves extracts of *M. hirsutum* on human B-lymphoma cells
2. To evaluate the selective cytotoxicity of these extracts on tumor cells.
3. To study the apoptotic induction activity of these extract on human-B lymphoma cells.
4. To investigate the effects of these extract on the cell cycle pattern of human B-lymphoma cells.
5. To elucidate the mechanisms of actions of these extracts on apoptotic induction and cell cycle arrest in human B-lymphoma cells.

Hypothesis

The branches and leaves of *M. hirsutum* contain compounds that have cytotoxicity on human B-lymphoma cells by apoptotic induction and/or cell cycle arrest.

Expected benefits and applications

The results from this study may clarify the potential cytotoxic effect of *M. hirsutum* on cancer cells. The information from this study may be benefit for further investigation of compounds from this plant in animal research or in clinical trial in the future.

Keywords

Micromelum hirsutum / cytotoxicity / apoptosis / cell cycle arrest