CHAPTER I

INTRODUCTION

Cancer is a group of diseases with the high incidence and death rate. A number of mechanisms, including overgrowing abnormal cells, have been illustrated for cancer formation. The abnormal cells causing cancer can be found in not only solid organs but also body fluid including blood and immune systems. One of intractable cancers is leukemia which is a blood cancer, characterized by involvement of abnormality of blood and bone marrow. According to the data collected in 2011, leukemia is one of the top-ranked, that causes death approximately 4.37 % of Thai people (Attasara and Buasom 2012). Generally, white blood cells or leukocytes are involved in protecting body from infectious diseases and foreign materials, resulting in normal body function. The overgrown white blood cells produced in the bone marrow, result in not only the unusual increment of cell proliferation but also the function abnormality. Moreover, the over-produced immature white blood cells lead to inhibition of other blood cells production. Thus, leukemia patients are lack of red blood cells and blood platelets, important factors for oxygen transportation and blood clotting processes, resulting in symptoms of difficulty breathing, anemia, bleeding, weakness, and frequent infections (National Cancer Institute, Department of Health and Human Services, 2013).

Bone marrow transplantation and chemotherapy are the two approaches for the treatment of leukemia. The bone marrow transplantation is a destructive procedure to patient's tissue with additional disadvantages including high cost of

treatment, possible further complications and limitation of accessibility. Alternatively, chemotherapy is the approach which uses chemotherapeutic agents to treat the abnormal cells. One of chemotherapeutic agents for leukemia is vincristine, a monoterpene indole alkaloid from a medicinal plant *Catharanthus roseus* (Cragg *et al.*, 2011). In addition, natural antileukemic substances can be isolated from endophytic fungi; for example brefeldin A from *Aspergillus clavatus* and *Paecilomyces* sp. (Wang *et al.*, 2002), dactylariol from *Pleospora* sp. IFB-E006 (Ge *et al.*, 2005), and penicillenone from *Pennicillium* sp. (Lin *et al.*, 2008).

Endophytic fungi are symbiotic microorganisms living within tissues of the plant hosts without causing noticeable diseases and well-known as rich sources of bioactive secondary metabolites (Strobel *et al.*, 2004). From the screening of endophytic fungi isolated from Chinese medicinal plants showed that 13.4 % of 172 endophytes produced substances with antileukemic activity against HL-60 cell line (Huang *et al.*, 2001). In this study, we were able to isolate an endophytic fungus *Phomopsis* sp. AANN8 from the twigs of the Thai medicinal plant *Artemisia annua* L. (Family Asteraceae), following by fermentation and preparation of the crude ethyl acetate extract from the fungal broth. From preliminary experiments, the final concentration at 20 µg/ml of the crude ethyl acetate extract was evaluated for cytotoxicity using four cell lines, including breast cancer (MCF-7), liver hepatocellular carcinoma (HepG2), human acute monocytic leukemia (THP-1), and African green monkey kidney (Vero) cell lines. The crude ethyl acetate extract exhibited cytotoxicity against these cell lines at -6.18 %, 9.04 %, 90.70 %, and -2.28 %, respectively. This finding suggested that the crude ethyl acetate extract might contain selective and strong antileukemic substances against THP-1 cells.

Interestingly, an EC_{50} of the ethyl acetate extract was 4.69 µg/ml that is relatively close to that of the positive control, ellipticine (EC_{50} at 3.37 µg/ml). Therefore, a study of secondary metabolites from this endophytic fungus and their antileukemic activity should be very motivating and informative for antileukemic drug development.

The purposes of this research were as follows:

1. Isolation and purification of compounds from the fermentation broth of the endophytic fungus *Phomopsis* sp. AANN8 by antileukemic assay-guided fractionation.
2. Determination of chemical structures of the isolated compounds.
3. Evaluation of the isolated compounds for antileukemic activity.