CHAPTER I

INTRODUCTION

Plants in the genus *Dendrobium* (Orchidaceae) have been used in traditional Chinese medicine as tonics to nourish the stomach, reduce fever and promote the production of body fluid (Zhang *et al.*, 2005). Many phytochemical studies of *Dendrobium* plants have been reported, and their main chemical constituents can be classified as bibenzyls, phenanthrenes, fluorenones, sesquiterpenes, flavonoids, coumarins and alkaloids (Hossain, 2011).

Several biological studies of *Dendrobium* plants have been reported, for example, free radical scavenging (Sritularak, Duangrak, and Likhitwitayawuid, 2011b), antimutagenic (Miyazawa *et al.*, 1997), anti-angiogenesis (Gong *et al.*, 2004), antiplatelet aggregation (Chen *et al.*, 1994), anti-inflammatory (Hwang *et al.*, 2010), antibacterial (Venkateswarlu, Raju, and Subbaraju, 2002), immunomodulatory (Zhao *et al.*, 2001) and neuroprotective (Wang *et al.*, 2010) activities.

Cancer is one of the leading causes of death in Thailand. It is actually a group of diseases involving unregulated cell growth. Cancer cells can invade nearby parts of the body and may also spread to distant parts through the lymphatic system or bloodstream. The causes of cancer are diverse, complex, and only partially understood. Many things are known to increase the risk of cancer, for instance, infections, genetics, radiation, obesity, smoking and environment pollutants. Cancer is usually treated with chemotherapy, radiation therapy and surgery (National Cancer Institute, 2008). Now, the search for new anti-cancer drugs with less side effects from the plants has received much attention. Up to the present, several cytotoxic constituents from *Dendrobium* spp. have been reported, for example, erianin, densiflorin and denchrysan A from *D. chrysotoxum* (Chen *et al.*, 2008b; Li, Wang, and Liu, 2001), crepidation and chrysotoxine from *D. capillipes* (Phechrmeekha, Sritularak, and Likhitwitayawuid, 2012), and moscatilin from several species of *Dendrobium*. (Kowitdamrong *et al.*, 2013). It is known that the development of metastasis is

a major cause of death for cancer patients. The processes of metastasis consist of cell migration, cell invasion and cell attachment. The invasion process permits neoplastic cells to enter the blood circulation and spread to lymph nodes, or systemically form secondary tumors in distinct organs. In this study, we aimed to investigate the apoptosis and anoikis effects of plant secondary metabolites. Apoptosis is a morphological phenomenon, including chromatin condensation and nuclear fragmentation (pyknosis), plasma membrane blebbing and cell shrinkage. Finally, the cell breaks into small membrane-surrounded fragments (apoptotic bodies), which are cleared by phagocytosis without an inflammatory response. The main mechanism of action of current anti-cancer drugs is to induce apoptosis of the cancer cells (Zhang *et al.*, 2012). Apoptosis machinery is a critical mechanism of the body to eliminate harmful cells. The cell will die from the mechanism inside and cause no effect to the surrounding cells (Reed, 2000). Unlike apoptosis, necrosis or accidental cell death is mediated by strong toxia or trauma that directly lyses cells. Necrosis will release cellular substances to the extracellular environment, resulting in the activation of the immune system as well as inflammation (Golstein and Kroemer, 2007). Together, apoptosis is the mode of action accepted as an important and safe way to eliminate cancer cells in human. Therefore, the apoptosis induction activity of the compounds would be investigated in the present study. Anoikis, a Greek word meaning "homeless", is apoptosis induced by loss of cell adhesion. Adhesion on the extracellular matrix (ECM) is important to determine whether a cell is in the correct location and to remove displaced cells by apoptosis. The physiological relevance of anoikis is confirmed by the fact that cancer cells, rather than normal epithelial cells, are usually not sensitive to anoikis which follows loss of cell anchorage, meaning the cells do not require adhesion to ECM to proliferate and survive. Anoikis resistance has been recognized as an important factor for success in metastasis (Hanahan and Weinberg, 2000; Mori *et al.*, 2009; Shanmugathasan and Jothy, 2000). A previous report showed anti-metastatic activity on lung cancer H23 cells of bibenzyls from *D. pulchellum* (Chanvorachote *et al.*, 2013).

Herpes is an infection that is caused by herpes simplex virus (HSV). There are two types of HSV, HSV-1 and HSV-2. HSV-1 is the main cause of herpes infections on

the mouth and lips, including cold sore and fever blisters. HSV-2 is the usual cause of genital herpes. After the first outbreak, the virus persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. The virus in a nerve cell become active again when something triggers them, for example, stress, illness, fever, sun exposure, menstrual periods and surgery. Now, drug resistant strains of HSV frequently increase following therapeutic treatment. Resistance to acyclovir and related nucleoside analogues can occur following mutation in either HSV thymidine kinase (TK) or DNA polymerase (Khan *et al.*, 2005). Previous studies revealed the anti-herpes simplex virus activity of several classes of plants metabolites such as phenylpropanoids, alkaloids, flavonoids, bibenzyls, terpenoids and anthraquinones (Chattopadhyay and Khan, 2008). A previous report disclosed that some compounds from *Dendrobium* plants showed anti-herpes simplex virus activity, for example, dendrofalconerols A and B from *D. falconeri* (Sritularak and Likhitwitayawuid, 2009).

Dendrobium plants are represented by more than 1,100 species, distributed throughout Asia, South East Asia and Australia (Martha, 2010). There are about 150 species of *Dendrobium* in Thailand (Seidenfaden, 1997). The plants of *Dendrobium* in Thailand according to Smitinand (2001) are as follows:

Dendrobium acerosum Lindl.	**กล้วยไม้มือนาง** Kluai mai mue nang (Chumphon)
D. acinaciforme Roxb.	**เอื้องยอดสร้อย** Ueang yot soi (Northern)
D. albosanguineum Lindl.	**เอื้องตางัว** Ueang ta ngua (Mae Hong Son)
D. aloifolium (Blume) Rchb.f.	**เอื้องมณี** Ueang mani (Bangkok)
D. anosmum Lindl.	**เอื้องสาย** Ueang sai (Chiang Mai, Peninsular)
D. aphyllum (Roxb.) C.E.C.Fisch.	**เอื้องงวงช้าง** Ueang nguang chang (Mae Hong Son)
D. bellatulum Rolfe	**เอื้องแซะภู** Ueng sae phu
D. bicameratum Lindl.	**เอื้องเข็ม** Ueang khem (Northern)

D. bilobulatum Seidenf.	**กล้วยไม้ก้างปลา** Kluai mai kang pla (General)
D. binoculare Rchb.f.	**เอื้องคำสาย** Ueang kham sai (Northern)
D. brymerianum Rchb.f.	**เอื้องคำฝอย** Ueang kham foi (Northern)
D. capillipes Rchb.f.	**เอื้องคำกิ่ว** Ueang kham kio (Lampang, Phrae)
D. cariniferum Rchb.f.	**เอื้องกาจก** Ueang kachok (Chiang Mai)
D. christyanum Rchb.f.	**เอื้องแซะภูกระดึง** Ueang sae phu kradueng (Loei)
D. chrysanthum Lindl.	**เอื้องสายมรกต** Ueang sai morakot (Bangkok)
D. chrysotoxum Lindl.	**เอื้องคำ** Ueang kham (Northern)
D. compactum Rolfe ex Hackett	**เอื้องข้าวตอก** Ueang khao tok (Northern)
D. concinnum Miq.	**หางเปีย** Hang pia (Narathiwat)
D. crepidatum Lindl. & Paxton	**เอื้องสายน้ำเขียว** Ueang sai nam khiao (General)
D. crocatum Hook.f.	**เอื้องนางนวล** Ueang nang nuan (Peninsular)
D. cruentum Rchb.f.	**เอื้องนกแก้ว** Ueang nok kaeo (Bangkok)
D. crumenatum Sw.	**หวายตะมอย** Wai tamoi (Central, Peninsular)
D. crystallinum Rchb.f.	**เอื้องนางฟ่อน** Ueang nang fon (Chiang Mai)
D. cumulatum Lindl.	**เอื้องสายสี่ดอก** Ueang sai si dok (Northern, Southeastern)
D. dantaniense Guillaumin	**เอื้องเข็ม** Ueang khem (Chiang Mai)
D. densiflorum Lindl.	**เอื้องมอนไข่** Ueang mon khai (Northern)
D. devonianum Paxton	**เอื้องเมี่ยง** Ueang miang (Chiang Mai)
D. dickasonii L.O. Williams	**เอื้องเคี้ยะ** Ueang khia (Chiang Mai)
D. discolor Lindl.	**หวายกลัก** Wai klak (Bangkok)

D. dixanthum Rchb.f.	**เอื้องเทียน** Ueang thian (Northern)
D. draconis Rchb.f.	**เอื้องเงิน** Ueang ngoen (Northern)
D. ellipsophyllum Tang & Wang	**เอื้องทอง** Ueang thong (General)
D. exile Schltr.	**เอื้องเสี้ยน** Ueang sian (General)
D. falconeri Hook.	**เอื้องสายวิสูตร** Ueang sai wisut (Bangkok)
D. farmeri Paxton	**เอื้องมัจฉาณุ** Ueang mat chanu (Bangkok)
D. fimbriatum Hook.	**เอื้องคำน้อย** Ueang kham noi (Chiang Mai)
D. findloyanum Parish & Rchb.f.	**พวงหยก** Phuang yok (Bangkok)
D. formosum Roxb. ex Lindl.	**เอื้องเงินหลวง** Ueang ngoen luang (Chiang Mai)
D. friedericksianum Rchb.f.	**เอื้องเหลืองจันทบูร** Ueang Lueang chantabun (Bangkok)
D. fuerstenbergianum Schltr.	**เอื้องแซะภูกระดึง** Ueang sae phukradueng (Loei)
D. gibsonii Lindl.	**เอื้องคำสาย** Ueang kham sai (Northern)
D. grande Hook.f	**เอื้องแผงใบใหญ่** Ueang pheang bai yai (Peninsular)
D. gratiosissimum Rchb.f.	**เอื้องกิ่งดำ** Ueang king dam (Bangkok)
D. gregulus Seidenf.	**เอื้องมะต่อม** Ueang matom (Chiang Mai)
D.griffithianum Lindl.	**เอื้องมัจฉาณุ** Ueang matchanu (Bangkok)
D. harveyanum Rchb.f.	**เอื้องคำฝอย** Ueang kham foi (Chiang Mai)
D. hendersonii Hawkes & Heller	**หวายตะมอยน้อย** Wai tamoi noi (Peninsular)
D. hercoglossum Rchb.f.	**เอื้องดอกมะเขือ** Ueang dok ma kuea (Bangkok)
D. heterocarpum Lindl.	**เอื้องสีตาล** Ueang si tan (Chiang Mai)

D. indivisum (Blume) Miq. var. *indivisum*	**ตานเสี้ยนไม้** Tan sian mai (Chumphon)
D. indivisum (Blume) Miq. var. *pallidum* Seidenf.	**ก้างปลา** Kang pla (General)
D. infundibulum Lindl.	**เอื้องตาเหิน** Ueang ta hoen (General)
D. intricatum Gagnep.	**เอื้องชมพู** Ueang chom phu (Chanthaburi)
D. jenkinsii Wall. ex Lindl.	**เอื้องผึ้งน้อย** Ueang phueng noi (Chiang Mai)
D. kanburiense Seidenf.	**หวายเมืองกาญจน์** Wai muang kan (Kanchanaburi)
D. leonis (Lindl.) Rchb.f.	**เอื้องตะขาบใหญ่** Ueang ta khap yai (General)
D. lindleyi Steud.	**เอื้องผึ้ง** Ueang phueng (Northern)
D. lituiflorum Lindl.	**เอื้องสายม่วง** Ueang sai muang (Bangkok, Northern)
D. moschatum (Buch.-Ham.) Sw.	**เอื้องจำปา** Ueang champa (Northern)
D. nathanielis Rchb.f.	**เกล็ดนิ่ม** Klet nim (Chantaburi)
D. nobile Lindl.	**เอื้องเค้าก่ิว** Ueang khao kio (Northern)
D. ochreatum Lindl.	**เอื้องตะขาบ** Ueang ta khap (Chiang Mai)
D. oligophyllum Gagnep.	**ข้าวตอกปราจีน** Khao tok prachin (General)
D. pachyglossum C.S.P.Parish & Rchb.f	**เอื้องขนหมู** Ueang khon mu (Mae Hong Son)
D. pachyphyllum (Kuntze) Bakh.f.	**เอื้องน้อย** Ueang noi (General)
D. palpebrae Lindl.	**เอื้องมัจฉา** Ueang mat cha, **เอื้องมัจฉาณุ** Ueang mat chanu (Bangkok)

D. parcum Rchb.f.	**เอื้องก้านกิ่ว** Ueang kan kio (Bangkok)
D. porishii Rchb.f.	**เอื้องครั่ง** Ueang khrang (Northern)
D. pendulum Roxb.	**เอื้องไม้เท้าฤาษี** Ueang mai thao ruesi (Bangkok, Chiang Mai)
D. pensile Ridl.	**หวาย** Wai (Narathiwat)
D. porphyrophyllum Guillaumin	**เอื้องลิ้น** Ueang lin (Lampang)
D. primulinum Lindl.	**เอื้องสายประสาท** Ueang sai prasat (Bangkok)
D. pulchellum Roxb. ex Lindl.	**เอื้องคำตาควาย** Ueang kham ta khwai (Mae Hong Son)
D. pychnostachyum Lindl.	**เศวตสอดสี** Sawet sot si (Chiang Mai)
D. salaccense (Blume) Lindl.	**เอื้องใบไผ่** Ueang bai phai (Chiang Mai)
D. scabrilingue Lindl.	**เอื้องแซะ** Ueang sae (Mae Hong Son)
D. secundum (Blume) Lindl.	**เอื้องแปรงสีฟัน** Ueang preang si fan (Bangkok)
D. seidenfadenii Rchb.f.	**เอื้องเก๊ียะ** Ueang kia (Chiang Mai)
D. senile Parish & Rchb.f.	**เอื้องชะนี** Ueang chani (Bangkok)
D. signatum Rchb.f.	**เอื้องเค้ากิ่ว** Ueang khao kio (Chiang Mai)
D. stuposum Lindl.	**เอื้องสาย** Ueang sai (Chiang Mai)
D. sulcatum Lindl.	**เอื้องจำปาน่าน** Ueang champa nan (Bangkok)
D. superbiens Rchb.f.	**หวายคิง** Wai khing (Bangkok)
D. sutepense Rolfe ex Downie	**เอื้องมะลิ** Ueang mali (Chiang Mai)
D. terminale Parish & Rchb.f	**เอื้องแผงโสภา** Ueang phaeng sopha (Peninsular)

D. thyrsiflorum Rchb.f	**เอื้องมอนไข่ใบมน** Ueang mon khai bai mon (Northern)
D. tortile Lindl.	**เอื้องไม้ตึง** Ueang mai tueng (Mae Hong Son)
D. trigonopus Rchb.f.	**เอื้องคำเหลี่ยม** Ueang kham liam (Chiang Mai)
D. trinervium Ridl.	**เทียนลิง** Thian ling (Chumphon)
D. unicum Seidenf.	**เอื้องครั่งแสด** Ueang krang saet (General)
D. uniflorum Griff.	**เอื้องทอง** Ueang thong (Pattani)
D. venustum Teijsm. & Binn	**ข้าวเหนียวลิง** Khao niao ling (Central)
D. villosulum Lindl.	**กล้วยหญ้านา** Kluai ya na (Bangkok)
D. virgineum Rchb.f.	**เอื้องเงินวิลาศ** Ueang ngoen wilat (Northern)
D. wardianum Warner	**เอื้องมณีไตรรงค์** Ueang mani trai rong (Northern)
D. wattii (Hook.f.) Rchb.f.	**เอื้องแซะ** Ueang sae (Northern)
D. ypsilon Seidenf.	**เอื้องแบนปากตัด** Ueang baen pak tat (General)

Dendrobium ellipsophyllum Tang & Wang is known in Thai as Ueang Thong (Smitinand, 2001). The plant has been found in Thailand, Burma, Laos, Vietnam and Yunnan Province China. It grows in evergreen lowland and primary montane forests. Its stems are cane-like, ridge with 20-40 cm length and 0.8-1 cm diameter. It has bilobed, elliptic, disticous and twisted leaves. The characteristics of flowers are single flowered influorescence with 1-2 cm sized and arise at opposite the leaves near apex of the canes. The petals and sepals are white which have dark yellow lips with brown lines at the middle. The flowering period is between June and August (The Botanical Garden Organization, 2011).

From the literature review, *Dendrobium ellipsophyllum* has never been studied for chemical constituents and bioactivities. Some fractions of the methanol extract of this plant were found to possess cytotoxic against human oral cavity

cancer cell. In addition, several compounds from *Dendrobium* plants were shown to posses anti-HSV activity. This study was therefore focused on the cytotoxic, anti-metastatic and anti-herpes simplex virus activities of isolated compounds from *Dendrobium ellipsophyllum*. The phytochemical data obtained in this study would contribute to the knowledge on the chemotaxonomy of this plant family and would provide valuable information for the biological studies of Thai medicinal plants.

The main objectives of this research are as follows:

1. Isolation and purification of chemical constituents from *Dendrobium ellipsophyllum*
2. Structure determination of isolated compounds
3. Evaluation of cytotoxic, anti-metastatic and anti-herpes simplex simplex virus activities of isolated compounds

Figure 1 *Dendrobium ellipsophyllum* Tang & Wang