CHAPTER I
INTRODUCTION

1.1 Background

People nowadays are more aware on health issue due to the emergence of new diseases. There is an intense demand for new therapies to treat and prevent these life-threatening diseases. Scientific and research interest is drawing its attention towards naturally-derived compounds as they are considered to have less toxic side effects compared to current treatments such as chemotherapy (Greenwell and Rahman, 2015). The genetic diversity of plants has provided not only for survival, but also a higher degree of comfort and the most important thing of all is the potential treatment for various diseases (Newman and Cragg 2016).

Research into the isolated plant constituents is great importance for the development of bioactive substances. The use of herbal medicines in Asia, Europe, and South America represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat many diseases. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported and thousands of phytochemicals from plants are safe and broadly effective alternatives with less adverse effect. Moreover, about 80% of the world population relies on traditional medicines for their primary health care needs. Around 1700 species have been documented for their biological properties and drug action and data is available for approximately 1200 species (Kong, Li, and Zhang, 2009).

In recent years, there is an increasing interest in searching antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases and retard lipid oxidative rancidity in foods (Diplock, 1998). Free radicals are highly reactive chemicals (ROS, RNS) that have the potential to harm cells. They are created when an atom or a molecule either gains or loses an electron. Free radicals are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals,

especially the damage to DNA, may play a role in the development of cancer and other health conditions (Valko *et al*., 2007).

To date there are various antioxidant activity assays, each one having their specific target within the matrix and all of them with advantages and disadvantages (Carocho and Ferreira, 2013). DPPH is a rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). It is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom (Krishnaiah, Sarbatly, and Nithyanandam, 2011).

Diabetes mellitus (DM) is the common endocrinal disorder and it is rapidly increasing disease in the human population all over the world. The numbers concerning the prevalence of DM are alarming; about 415 million people worldwide are estimated to have diabetes, expected that the numbers will rise to 642 million or more diabetic patients in 2040 (Gothai et al., 2016). Diabetes Mellitus can be classified into clinical categories as: type 1 Diabetes (T1DM), caused by β-cell destruction and usually leading to absolute insulin deficiency, type 2 Diabetes (T2DM), due to a defect on the background of insulin resistance, and others such as gestational diabetes and specific types such as monogenic diabetes syndromes, exocrine pancreas diseases, and drug or chemical-induced diabetes (Challa, 2014; Munhoz, 2018). Although obesity and physical inactivity are known to be major risk factors for type 2 diabetes (T2DM), recent evidence suggests that oxidative stress may contribute to the pathogenesis of T2DM by increasing insulin resistance or impairing insulin secretion (Bajaj and Khan, 2012). Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decreases antioxidant potential which, leads to disturbances in the balance between radical formation and protection against which ultimately results in oxidative damage of cell components such as proteins, lipids, and nucleic acids. An increased oxidative stress can be observed in both insulin-dependent (type 1) and non-insulin-dependent diabetes (type 2) (Sindhi *et al*., 2013). Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes. One of the established therapeutics to treat type 2 diabetes is to control blood glucose levels after eating. Decreased blood glucose levels after eating can be done with delaying the absorption of glucose by inhibiting the enzyme α-glucosidase

activity (Cahyani and Purwaningsih, 2015). In recent years, some of the standard synthetic drugs used for the treatment of diabetes lead to cause side effects like nausea, vomiting, abdominal pain, diarrhea, head ache, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention or swelling. Moreover, they are not safe for use during pregnancy. Active research has been performed on traditional available medicinal plants for discovery of new antidiabetic drug as an alternative for synthetic drugs (Abirami, Natarajan, and Sagadevan, 2014).

Cancer is the second leading cause of death worldwide. Cervical and breast cancer are the most dead list diseases among women, both in developed and developing countries. Cervical cancer can occurred when the abnormal cells are undergoing to the rapid and uncontrolled growth on the cervix. Current treatments for cervical cancer may include surgery, drugs (hormonal therapy and chemotherapy), radiation and/or immunotherapy. Conventional cancer treatments such as chemotherapy and radiotherapy have shown some effectiveness for reducing or eradicating cancers; however, they can produce unpleasant side effects, e.g. nausea, vomiting, changes in bowel habits, fatigue and hair loss. Several studies have confirmed the anti-proliferative and cell cycle regulatory effects of some plants which behave as cancer prevention (Amin *et al*. 2009; Azarifar *et al*. 2015). More than 25% of drugs used during the last 20 years are directly derived from plants, while the other 25% are chemically altered natural products. Still, only 5-15% of the approximately 250,000 higher plants have ever been investigated for bioactive compounds. The advantage of using such compounds for cancer treatment is their relatively non-toxic nature and availability in an ingestive form (Amin *et al*. 2009).

Geographically, Myanmar (formerly Burma) is situated on the dividing line between the Indian sub- continent and South- east Asia, and holds incredibly rich biodiversity and habitats. In Myanmar, valuable medicinal plants are found abundantly, most of them have not been scientifically investigated yet. Currently, the root parts of *C. excavata* were selected because of their widely used in traditional remedies in Myanmar such as, to treat headache, itching, flu, snake-bite and detoxification.

The genus *Clausena* belongs to the Rutaceae family, comprises of about 14 species of evergreen trees (Ismail et al., 2012). *C. excavata* is one the most well-known species in genus *Clausena,* is a shrub with strong and rather objectionable smell, found

from the Himalayas and China to and throughout Southeast Asia (Taufiq-Yap *et al.*, 2007; Arbab et al., 2013). In Myanmar, it is locally known as "Sat pu Khar yar", "Taw Pyin Daw Thein". *Clausena excavata* is famous among phytochemists and pharmacologists for its abundant source of secondary metabolites, especially carbazole alkaloids, coumarins (furano and pyrano), few flavonoids and lemonoids (Wu et al., 1999; Thuy et al., 1999; Ito et al., 2000;Taufiq-Yap et al., 2007; Seo et al., 2017; Liu *et al.*, 2018). Many pharmacologically active compounds isolated from this species have been used for the treatment of human diseases such as cardiovascular disease, antiinflammatory**,** antioxidants, anti-snake venom, anticancer, anti-HIV, and antiplatelet (Auranwiwat et al., 2014; Arbab et al., 2015; Chakthong *et al.*, 2016; Huang et al., 2017; Ma *et al.*, 2017). Several parts of the plant have been used as a traditional medicine for the treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, and snake-bite and detoxification agents in Thailand and Southeast Asia country. Scientifically many novel bioactive compounds have been isolated and reported from different parts of the plant, but there has not been a report yet from Myanmar *C. excavata*. In addition, *Clausena* species has been found to possess antidiabetic activity (Damsud, Chanwun, and Kaewpiboon, 2017).

In this present research work, the secondary metabolites were isolated from Myanmar medicinal plant (*C. excavata*). Moreover, modification of TMT-1 (nordentatin) afforded pyranocoumarin benzoate compounds. Identified bioactive compounds were examined for antioxidant, antidiabetic, and anticancer activities and also their structural-activity relationship was studied. The plant sample was collected from Myanmar was carried out according to the natural product isolation procedures. Firstly, the dried root sample was extracted with solvent, according to standard protocols. The solvent extract was evaporated to remove the final traces of the respective solvents. The different compounds in pure form were isolated by using various chromatographic techniques. The isolation process of pure compounds was done by using column chromatography, and their characterization were recorded in advance spectroscopic methods; UV-vis, IR, (1D, 2D-NMR), DART-MS. Here in seven compounds, TMT-**1 (**nordentatin), TMT-**2 (**dentatin), TMT-**3** (heptaphylline), TMT-**5** (mukonine), TMT-**6 (**xenthoxylentin), TMT-**7** (7-hydroxyheptaphylline) including one new compound, TMT-**4** (excavatin-A) were isolated from *C. excavata* and ten new

compounds, TMT-**1a** to TMT-**1j** were semi-synthesized from TMT-**1** (nordentatin). The antioxidant activity of identified compounds was tested by DPPH assay, antidiabetic activity was carried out by α-glucosidase assay, and anticancer activity was performed by MTT assay.

1.2 The Problems of Research

- What are the secondary metabolites from the roots of Myanmar medicinal plant (*C. excavata*)?
- How are the antioxidant, antidiabetic, and anticancer activities of isolated and modified compounds, from *C. excavata*?
- How is the between molecular structure-activity relationship of isolated and modified compounds?

1.3 The Purposes of Research

- To isolate and modify the secondary metabolites from the roots of Myanmar medicinal plants (*C. excavata*).
- To evaluate antioxidant, antidiabetic, and anticancer activities of isolated and modified compounds from *C. excavata* by using DPPH, α-glucosidase and MTT assay.
- To investigate the relationship between molecular structure of isolated compounds and their antioxidant, antidiabetic and anticancer activities.

1.4 Benefit of Research

This current research will report phytoconstituents and its modified compounds that to be useful for drug lead discovery of antioxidant, antidiabetic, and anticancer agents. The evaluation of relationship between molecular structure of isolated and modified compounds and their activities may support researchers who are searching potential natural origin drugs.