CHAPTER 5

Conclusions

Graptophyllum pictum (L.) Griff. is a Papua New Guinea native shrub which is widely used in traditional medicine in New Guinea and Asia. *G. pictum* has been used to relieve earache, and as a diuretic and an antipyretic. The chemical constituents found in this plant were vomifoliol and flavonoids. The biological activities of the plant extracts have been reported to be anti-inflammatory, to reduce blood glucose levels and have antibacterial activity.

In this study, the dried leaves of *G. pictum* were macerated in hexane, $CHCl_3$ and MeOH, and in hexane, CH_2Cl_2 and EtOH. In addition, the leaves were also extracted with 95% EtOH and then partitioned with hexane**,** EtOAc and *n*-BuOH, respectively. The fresh leaves of *G. pictum* was hydrodistilled using a modified Clevenger-type apparatus to obtain a yellow oil which was further analyzed by GC-FID and GC-MS. The major components of this essential oil were phytol (77.7%), *n*-nonacosane (6.5%) and hexahydrofarnesyl acetone (2.6%).

The biological activities of all extracts, fractions and the essential oil were determined. The essential oil inhibited the growth of the KB (epidermoid carcinoma of oral cavity), NCI-H187 (small cell lung carcinoma) cell lines and the Vero cell line with IC_{50} values of 27.04, 25.27 and 26.52 µg/mL, respectively. Whereas, the 95% EtOH, hexane, EtOAc and aqueous fractions only inhibited the growth of MCF-7 (breast adenocarcinoma) cell lines with IC_{50} values of 33.27, 38.66, 26.01 and 20.41 µg/mL, respectively. The extracts and the fractions were non-cytotoxic against Vero cells.

The essential oil of *G. pictum* showed antibacterial activities against both *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). All extracts from the maceration method could inhibit *Pseudomonas aeruginasa* (*P. aeruginosa*) and also

inhibited fungi; *Aspergillus flavus* (*A. flavus*), *Candida albican* (*C. albican*) and *Trichophyton mentagrophyte* (*T. mentagrophyte*) using the agar diffusion assay.

All the extracts, fractions and the essential oil possessed antioxidant activities as was determined using the ABTS and DPPH methods. The total phenolic and flavonoid contents were also investigated. A preliminary phytochemical screening of *G. pictum* leaves revealed they contained flavonoids, steroids, tannins, coumarins, saponins, anthraquinones, phenolics and sugars.

The hexane fraction of *G. pictum* showed anticancer activity against MCF-7. Therefore, this fraction was selected for analysis using GC-MS and further isolation. The major components of the hexane fraction were squalene (23.8%), phytol (18.3%) and neophytadiene (13.4%). Whereas, two compounds, squalene and stigmasterol, could be isolated from this fraction. While, 1,4-diglycoloyl-benzene was also isolated from the EtOAc fraction. There are few publications related to *G. pictum*, thus this study has provided more information about *G. pictum*, which may be useful for further investigations.

Solanum spirale Roxb. is a small shrub which is widely distributed in Asia and Oceania. The traditional usages include the treatment of cough, stomachache and beriberi. The chemical constituents of *S. spirale* were reported as alkaloids, steroidal glycosides, lupeol, protocatechuic acid and *trans*-cinnamic acid. The major compounds in the essential oil from the leaves were found to be (*E*)-phytol, β-selinene and α-selinene. The biological screening of *S. spirale* showed antimicrobial, antioxidant and anticancer activities. Furthermore, the $CHCl_3$ extract of *S. spirale* leaves possessed anti-HSV-1 activity.

In this investigation, the dried stems of *S. spirale* were macerated in hexane, $CHCl_3$ and MeOH. From a previous biological activity study, the $CHCl_3$ extract was selected to analyze using GC-MS and further purification. The major components of the $CHCl_3$ extract were triacetin (16.1%), neophytadiene (7.4%) and ethyl hexadecanoate (6.3%). Scopoletin was isolated from the extract. This compound has been reported to possess many biological properties such as cytotoxicity, antioxidant and anti-

inflammatory activities. This study has provided additional information on *S. spirale* to assist further investigations in the future.

Gynura divaricata (L.) DC., a Chinese traditional medicinal herb, is widely distributed in Asia, including Thailand. The traditional uses of *G. divaricata* have been for the treatment of bronchitis, pulmonary tuberculosis and diabetes. Many chemical constituents of *G. divaricata* have been identified for example; sterols, flavonoids and triterpenes. The essential oil constituents from the leaves of *G. divaricata* in China have been also studied. Moreover, the biological activities of the plant extracts have been revealed such as their antioxidant, antimicrobial and anti-diabetic properties.

In this study, the dried leaves of *G. divaricata* were macerated in hexane, CH_2Cl_2 and MeOH, and also extracted using a Soxhlet extractor using petroleum ether, EtOAc and EtOH. Whereas, the fresh leaves of *G. divaricata* were hydrodistilled using a modified Clevenger-type apparatus to obtain a yellow oil which was further analyzed by GC-FID and GC-MS. The major components of this essential oil were cubenol (65.7%) and spathulenol (6.4%), which were different from the previous reports.

The cytotoxicity, anti-tuberculosis and antimicrobial activities of all the extracts and the essential oil of *G. divaricata* were investigated. The essential oil showed cytotoxicity against KB, MCF-7 and NCI-H187 cell lines with IC_{50} values of 5.79, 47.44 and 17.65 µg/mL, respectively. Both extracts and the essential oil were non-cytotoxic against Vero cells. In addition, this essential oil could inhibit *M. tuberculosis* H_{37}Ra with a MIC value of 50 µg/mL and showed antibacterial activity against *S. aureus*, *P. aeruginosa* and *E.coil*, while the extracts exhibited antibacterial activity only against *S. aureus* and *P. aeruginosa*. The extracts also possessed antifungal activities against *A. flavus*, *C. albican* and *T. mentagrophyte* using the agar diffusion assay. Furthermore, the essential oil and the extracts exhibited antioxidant activities.

Due to the anticancer and antimycobacterial activity of the essential oil of *G. divaricata*, this essential oil was further isolated and purified to afford the major components, cubenol and spathulenol. The cytotoxic activities of these two compounds were determined against KB, MCF-7 and NCI-H187 cell lines and their antimycobacterial activities against *M. tuberculosis* H37Ra. Only cubenol could inhibit

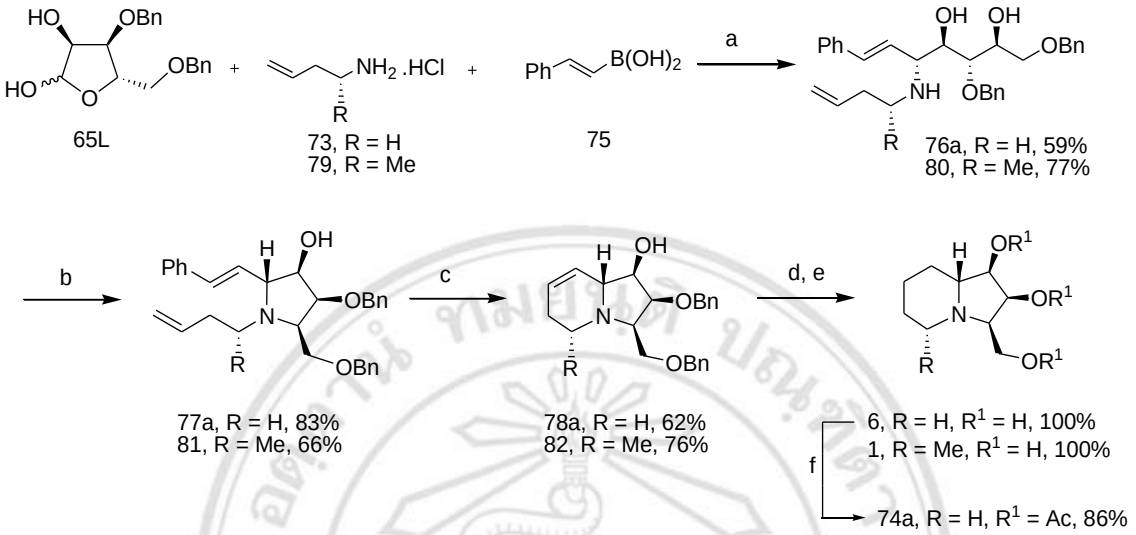
the NCI-H187 cell line with an IC_{50} value of 45.37 µg/mL. This is the first report on the biological activity and the isolation of the essential oil of *G. divaricata*. The information from this study will be useful for further drug discovery from *G. divaricata*.

CHAPTER 5

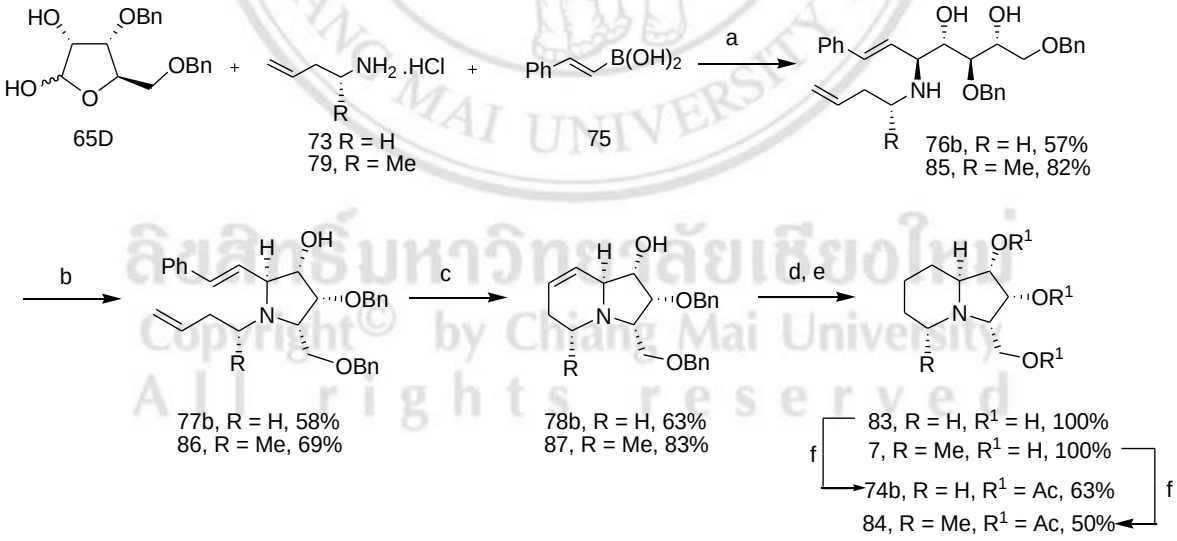
Conclusions

The development of a new concise synthetic strategy for the preparation of natural (−)-steviamine and its analogues was achieved in four synthetic steps from readily available β-L and β-D ribofuranose derivatives. This synthetic protocol involves a highly anti-selective Petasis reaction, a selective *O*-mesylate cyclization reaction, an efficient ring-closing metathesis reaction and hydrogenation reactions. The starting material, the β-L-ribofuranose derivative [**65L**], was prepared from commercially available β-L-ribofuranose 1,2,3,5-tetraacetate. It was prepared in four steps in 45% overall yield. This compound [**65L**] was used as a precursor in the above synthetic protocol to generate 10-*nor*-steviamine [**6**] and (−)-steviamine [**1**] in 30% and 39% overall yields, respectively (Scheme 5.1). The triacetate derivative of 10-*nor*-steviamine [**6**] was prepared following Scheme 5.1 giving the desired product [**74a**] in 86% yield. Furthermore, another starting material, the β-D-ribofuranose derivative [**65D**], was synthesized from β-D-ribofuranose 1,2,3,5-tetraacetate in 63% overall yield. The 10-*nor*-*ent*-steviamine [**83**] and 5-*epi*-*ent*-steviamine [**7**] were also prepared in an analogues fashion from **65D** in 21% and 47% overall yields, respectively (Scheme 5.2). The triacetate derivatives of 10-*nor*-*ent*-steviamine [**83**] and 5-*epi*-*ent*-steviamine [**7**] were synthesized to obtain compound **74b** and **84** in 63% and 50% yields, respectively (Scheme 5.2). All synthetic compounds were tested as glycosidase inhibitors against twelve glycosidases (at 143 μg/mL concentrations). The results showed that these compounds in general have poor to moderate inhibitory activity against most enzymes. The 10-*nor* analogues however, showed 50-53% inhibition of α-L-rhamnosidase from *Penicillium decumbens*, while 10-*nor*-steviamine [**6**] exhibited 51% inhibition of *N*-acetyl-β-D-glucosaminidase (from bovine kidney) at the same concentration (760 μM). The concise synthesis of (−)-steviamine [**1**] and its analogues and their glycosidase inhibitory activities in this study may be used as a guideline for the synthesis of other

polyhydroxylated indolizidine alkaloids which have potential glycosidase inhibitory activities for further new drug discovery.



Scheme 5.1. Reagents and conditions: (a) Et_3N, EtOH, rt, 4 d; (b) Et_3N, MsCl, CH_2Cl_2, -10 °C to 45 °C, 4 h; (c) $Ti(O^iPr)_4$, Grubbs' II catalyst, CH_2Cl_2, 45 °C, 2.5 h; (d) $PdCl_2$, H_2, MeOH, 3 h; (e) basic ion-exchange; (f) Ac_2O, pyridine, rt, 18 h.



Scheme 5.2. Reagents and conditions: (a) Et_3N, EtOH, rt, 4 d; (b) Et_3N, MsCl, CH_2Cl_2, -10 °C to 45 °C, 4 h; (c) $Ti(O^iPr)_4$, Grubbs' II catalyst, CH_2Cl_2, 45 °C, 2.5 h; (d) $PdCl_2$, H_2, MeOH, 3 h; (e) basic ion-exchange; (f) Ac_2O, pyridine, rt, 18 h.