CHAPTER 5

Conclusion

Four selected *Combretum* species including, *Combretum deciduum* Coll. & Hemsl., *Combretum griffithii* Heur. & M.A., *Combretum latifolium* Bl. and *Combretum quadrangulare* Kurz were investigated their biological activities and chemical compositions.

Prior to this research, this is the first report that describes the chemical compositions of the leaf volatile compunds of *C. latifolium* Bl. and *C. quadrangulare* Kurz and their antibacterial activities. Also the first report of biological activity, including antibacterial activity, antifungal activity, anti-tuberculosis activity, antioxidant activity, anticancer activity and their cytotoxicity from the crude extracts of the leaves of *C. deciduum* Coll. & Hemsl., *C. griffithii* Heur. & M.A. and *C. quadrangulare* Kurz. Furthermore, this is the first report of the chemical constituents and their antibacterial activity from the leaves of *C. griffithii* Heur. & M.A. and *C. latifolium* Bl.

The goal of this research was to discover antibacterial substances from four selected *Combretum* species and was concerned with the isolation and structural elucidation of their secondary metabolites. The leaf extracts and volatile compounds from selected plants were subjected to bioassay screening for antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* All methanolic leaf extracts of four *Combretum* species exhibited potent antibacterial activity against *S. aureus* and *P. aeruginosa* than *n*-hexane and dichloromethane extracts, by using ampicillin, gentamicin and ceftriazone as the reference standards. The leaf volatile compounds of *C. latifolium* Bl. and *C. quadrangulare* Kurz showed antibacterial activity against *S. aureus* and *E. coli*. All the crude extracts of four *Combretum* species leaves showed antifungal activity against *Aspergillus flavas*. All the

crude extracts of *C. deciduum* Coll. & Hemsl. and methanolic extract of *C. quadrangulare* Kurz showed antifungal activity against *Candida albicans*. While, all crude extracts of leaves of four *Combretum* species showed inactive in antifungal activity against *Trichophyton mentagophyte*. And all the crude extracts of four *Combretum* species leaves showed no anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Ra. The antioxidant activity of the crude leaf extracts of each plant were determined using the ABTS and DPPH methods. The results were compared with antioxidant standard, Trolox, vitamin C and quercetin. All methanolic extract of four *Combretum* species leaves exhibited antioxidant activity (ABTS and DPPH method), followed by *n*-hexane and dichloromethane extracts. The methanolic leaf extract of *C. latifolium* Bl. exhibited the highest antioxidant activity (ABTS and DPPH method). The anticancer activity of the selected active extracts (methanolic extracts) of four *Combretum* species leaves were performed using the resazurin microplate assay (REMA). The methanolic leaf extracts of all four *Combretum* species exhibited significant anticancer activity against KB (epidermoid carcinoma of the oral cavity), MCF7 (breast adenocarcinoma) and NCI-H187 (small cell lung carcinoma) cell lines. The methanolic leaf extract of *C. deciduum* Coll. & Hemsl. inhibited KB-oral cavity and MCF7-breast cancer cell lines. The methanolic leaf extract of *C. latifolium* Bl. inhibited MCF7-breast cancer cell lines. Methanolic leaf extract of *C. quadrangulare* Kurz inhibited KB-oral cavity and NCI-H187-small cell lung cancer cell lines. However, methanolic leaf extract of *C. griffithii* Heur. & M.A. was inactive against all three cell lines. Ellipticine, Doxorubicin and Tamoxifen were used as standard compounds. The cytotoxicity of the selected active extracts were performed using Green Fluorescent Protein (GFP) detection methodology. All methanolic extracts were non-cytotoxic against primate cell lines (Vero cells).

Preliminary bioassays in this work revealed that the extracts and the volatile compounds from these four selected *Combretum* species leaves showed interesting biological activities, including antibacterial, anticancer, cytotoxic and antioxidant activities. And the biological activities were used as a guide for the isolation, purification and structure elucidation of secondary metabolites.

The volatile compounds were analysed by GC-MS. The main components found in the leaf volatile fractions of *C. latifolium* Bl. and *C. quadrangulare* Kurz were palmitic acid (37.05% and 17.74%), with hexahydrofanesyl acetone (11.54% and 17.36%), isophytol (13.47% and 3.71%), neophytadiene (7.71% and 3.52%) and *n*-nonacosane (4.68% and 5.37%), respectively. Other major compounds of *C. latifolium* Bl. were phytol isomer (7.17%), and for *C. quadrangulare* Kurz oil were *β*-selinene (2.46%), tetradecanoic acid (1.54%), phytol (11.52%) and *n*-heptacosane (4.75%). The *C. latifolium* Bl. leaf volatile fraction showed potent antibacterial activity against selected strains of bacteria (*S. aureus* and *E. coli*) than *C. quadrangulare* Kurz. These results suggested that the volatile compounds of the leaves of *C. latifolium* Bl. and *C. quadrangulare* Kurz could be used for treatment of infectious diseases caused by *S. aureus* and *E. coli*. And further research is needed to get more information on other biological activities of these oils.

The investigation from the methanolic extract leaves of *C. griffithii* Heur. & M.A., 3*β*-taraxerol was isolated. Chemical examination of the methanolic extract leaves of *C. quadrangulare* Kurz led to the isolation of two compounds, namely 3*β*-taraxerol, along with lupeol. From the methanolic extract leaves of *C. latifolium* Bl., two lipid compounds were isolated. The lipid compounds were analysed by GC-MS. The main components found in two lipid compounds were the mixture of hydrocarbons, including *n*-octacotane (28.48%), docosane (14.53%), eicosane (8.30% and 4.89%), *n*-nonacosane (39.21%), *n*-heptacosane (14.71%) and *n*-heneicosane (3.25%). In addition, 3*β*-taraxerol, lupeol and these two lipids compounds showed antibacterial activity against *S. aureus* and *E. coli*.

They have been used of selected *Combretum* species, except *C. deciduum* Coll. & Hemsl. as a component of traditional medicines in Northern Thailand, Phrae Province, for several indications, however our study has supported these traditional uses for ailments such as diarrhoea sore through wounds. Therefore, the folk medicines usage can be supported the antibacterial activity of the isolated constituents and might be useful for new drug development. There appear to be many possibilities for use of *Combretum* derivative in ethnomedicine, continuing studies on ethnopharmacology are essential for the development of the medicines.