CHAPTER VI
CONCLUSION

The author has succeeded in the isolation of deoxypreussomerin derivatives, palmarumycins JC1 **[70]** and JC2 **[71]**, and two dimeric naphthoquinones, isodiospyrin **[23]** and its new derivative isodiospyrol A **[72]** from dried fruits of *Diospyros ehretioides* which were collected from Kaeng Tana National Park, Ubon Ratchathani province. Dried fruits of *Diospyros ehretioides* were macerated with CH_2Cl_2 and a crude extract was chromatographed on Sephadex LH-20 column (eluted with MeOH) and further purified by semi-preparative HPLC (reversed-phase, RP C_{18} column) eluted with MeCN/H_2O to obtain pure compound. The isolated compounds were subsequently elucidated for their chemical structures by spectroscopic analyses and their biological activities were investigated. Data in all appendices are real data without any single modification.

The research results in this dissertation have revealed some crucial findings in terms of isolation, structural elucidation and biological evaluation of the identified compounds. Palmarumycin JC1 **[71]** and isodiospyrin **[23]** did not exhibit antitubercular, antifungal and antimalarial activities in our bioassay systems. Interestingly, palmarumycin JC2 **[71]** displayed antimalarial (IC_{50} 4.5 μg/mL), antifungal (IC_{50} 12.5 μg/mL) and antitubercular (MIC 6.25 μg/mL) activities.

Moreover, isodiospyrol A **[72]** demonstrated antimalarial (IC_{50} 2.7 μg/mL) and antitubercular (MIC 50 μg/mL) activities, but was inactive towards *Candida albican*.

Moreover, further isolation of bioactive compounds from the CH_2Cl_2 wood extract of *Diospyros rhodocalyx* was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel which provided two sterols, β-sitosterol **[11]** and stigmasterol **[12]**; two triterpenes**,** lupeol **[5]** and betulinaldehyde **[67]**; and a naphthoquinone, diospyrin **[21]** while a CH_2Cl_2 wood extract of *Diospyros glandulosa* was sequentially subjected to column chromatography on Sephadex LH-20 and silica gel, obtaining lupeol **[5]**, β-sitosterol **[11]**, stigmasterol **[12]** and diospyrin **[21]**. Due to β-sitosterol **[11]** and stigmasterol **[12]** were not able to dissolve in DMSO, therefore, antimalarial and antimycobacterial activity tests were not evaluated. However, only lupeol**[5],** diospyrin **[21]** and betulinaldehyde **[67]** were subjected to antimalarial and antimycobacterial activity tests. Of all the isolated compounds, diospyrin **[21]** possessed the most potent antimalarial activity against *Plasmodium falciparum* K1 with the IC_{50} value of 3.29 μg/mL and antimycobacterial activity with the MIC value at 6.25 μg/mL

An interesting notification was that palmarumycins were not found in the extract of freshly-collected fruits. However, they were present in dried fruit extracts. Base on the absence of palmarumycins in fresh fruits of *Diospyros ehretioides*, together with the chemotaxonomic point of view, the author proposed that palmarumycins JC1 **[70]** and JC2 **[71]** are more likely to be epiphytic or endophytic fungal metabolites, not of the plant.

To clarify the origin of palmarumycin JC1**[70]** and JC2 **[71]**, both fresh and dried fruits of *Diospyros ehretioides* were re-collected. The fresh fruits were

macerated with MeOH, while the dried fruits were macerated with CH_2Cl_2 (in the same manner as that of the first batch). The fresh fruits extract was fractionated by Sephadex LH-20 column chromatography, and fractions were subjected to ^{1}H-NMR analysis for detection of signals for palmarumycins JC1 **[70]** and JC2 **[71]**. Palmarumycins were not found in the extract of freshly-collected fruits, however, they were present in dried fruit extract. Moreover, further study enabled the isolation of endophytic fungi from fresh fruits whilst epiphytic fungi were isolated from dried fruits. Endophytic and epiphytic fungi were grown on different culture media. Mycelia and broth of these fungal cultures were separately extracted with organic solvents. Their extracts were evaporated to dryness, subsequently subjected to ^{1}H-NMR analysis. However, palmarumycins were not detected in any crude extract of fungal cells and broth. Therefore, it is concluded that these fungal isolates could not produce palmarumycins or if they could produce, the production may be too low to be detected by ^{1}H-NMR analysis.

More work should be done such as optimizing different culture media. Moreover, using suitable organic solvents would be necessary for extraction to produce more fungal metabolites. Since palmarumycin JC2 **[71]** displayed antimalarial (IC_{50} 4.5 μg/mL), antifungal (IC_{50} 12.5 μg/mL) and antitubercular (MIC 6.25 μg/mL) activities, therefore, the improved production of JC2 **[71]** would be necessary for drug development, i.e., through tissue culture and synthesis.