CHAPTER 5

CONCLUSION

Three medicinal plants: *Acalypha indica* Linn., *Bridelia retusa* (L.) A. Juss. and *Cleidion javanicum* BL. (Euphorbiaceae family) were used in this investigation. They have been used as a component of traditional medicines for several indications in a number of countries, however little scientific evidence has supported these traditional uses. Preliminary bioassays in this work revealed that the extracts and the essential oils from these plants showed interesting biological activities, including antimicrobial, cytotoxic and antioxidant activities.

The dried plant powders from aerial parts of *A. indica*, leaves, stems and fruits of *B. retusa* and *C. javanicum* were successively macerated with hexane, chloroform, and methanol sequentially. The crude extracts of each plant were obtained for further investigation. The essential oil from the fresh aerial parts of *A. indica*, the fresh leaves, stems and fruits of *B. retusa* and *C. javanicum* were isolated by hydrodistillation and analysed by using a combination of GC (FID) and GC-MS.

The major components of *A. indica* were phytol (47.49%), 9-tricosene (7.05%), pentacosane (3.96%), nonacosane (1.61%), and heptacosane (1.23%). The major constituents of *C. javanicum* leaves were ethyl linoleolate (32.12%), hexadecanoic acid (26.77%), trans-phytol (24.64%) and iso-phytol (4.80%). The major constituents of *C. javanicum* fruits were hexadecanoic acid (34.42%), linoleic acid (21.68), 9, 12, 15-Octadecatrien-1-ol (21.00%), phthalic acid (4.64%) and phytol (1.19%). The major constituents of *B. retusa* leaves were phytol (33.4%), phthalic acid (5.2%), 6, 13-dimethoxy-2, 3, 9, 10-tetramethylpentacene-1, 4, 8, 11-tetrone (3.4%), heptacosane (2.3%) and nonacosane (1.2%). The major constituents of *B. retusa* fruits were dibutyl sebacate (25.6%), phytol isomer (4.8%), diacetin (4.3%), tricosane (3.9%), isophytol (2.7%), phthalic acid (1.9%), hexadecanoic acid (1.5%), and eicosane (1.2%).

Phytol, an acyclic diterpene alcohol was the most prominent compound which present in the leaves of three medicinal plants. It has been reported to possess anticancer, antibacterial and anti-tuberculosis activities. Phytol can be used as a precursor for the production of the synthesis forms of vitamin E and vitamin K, and also used in cosmetic products.

The antimicrobial activity of the crude extracts and the essential oil of each plant were determined by the agar diffusion method (in extracts) and disc diffusion method (in essential oils). The methanolic extracts of *C. javanicum* leaves, the chloroform extract of *B. retusa* fruits and the essential oils of all three medicinal plants exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, using gentamicin as the reference standard. Only the crude extracts of *A. indica* showed antifungal activity against *Candida albicans*, *Aspergillus flavas* and *Trichophyton mentagophyte*. Ketoconazole was used as the reference standard. The crude extracts of *B. retusa* and *C. javanicum* showed antifungal activity against only *A. flavas* and *T. mentagophyte*.

The MIC values of the selected active essential oils were investigated using disc diffusion method. The most active essential oil was the fruit essential oil of *C. javanicum* which showed antibacterial activity against all of three bacterials: *S. aureus*, *E. coli* and *P. aeruginosa* with MIC values of 10 mg mL^{-1}.

The antioxidant activity of the crude extracts and the essential oil of each plant were determined using the ABTS and DPPH methods. The results were compared with antioxidant standard, trolox. The essential oil of *C. javanicum* fruits exhibited the highest antioxidant activity (ABTS) followed by *B. retusa* leaves, *B. retusa* fruits, *A. indica* aerial parts and *C. javanicum* leaves, respectively. Only the methanol extract of *B. retusa* fruits exhibited antioxidant activity (ABTS). The essential oil of *C. javanicum* leaves exhibited the highest antioxidant activity (DPPH) followed by *A. indica* aerial parts respectively. The methanolic extract of *B. retusa* stems exhibited the highest antioxidant activity (DPPH).

The total phenolic contents of crude extracts of *A. indica*, *C. javanicum* and *B. retusa* in various solvents: hexane, chloroform and methanol were investigated. Total phenolic contents of the test samples were expressed as % gallic acid (w/w) of dry plant material by comparison with the gallic acid standard curve. The methanol extract

of *C. javanicum* stems exhibited the highest total phenol contents of the extracts. The total flavonoid contents of crude extracts of *A. indica*, *C. javanicum* and *B. retusa* in various solvents: hexane, chloroform and methanol were also investigated. Total flavonoid contents of the test samples were expressed as % quercetin (w/w) of dry plant materials by comparison with the quercetin standard curve. The chloroform extract of *A. indica* aerial parts exhibited the highest total flavonoid contents of the extracts.

The cytotoxicity activity of the selected active extracts and the selected active essential oils of *A. indica*, *C. javanicum* and *B. retusa* were performed using the resazurin microplate assay (REMA). The essential oil of *A. indica* aerial parts and the essential oil of *C. javanicum* leaves showed cytotoxicity activity against all of three cancer cell lines: KB-Oral cavity cancer, MCF7-Breast cancer and NCI-H187-Small cell lung cancer. Only the leaves methanol extract of *B. retusa* exhibited anticancer activity against all of three cancer cell: KB-Oral cavity cancer, MCF7-Breast cancer and NCI-H187-Small cell lung cancer.

The cytotoxicity of the selected active extracts and the selected active essential oils of *A. indica*, *C. javanicum* and *B. retusa* were performed using Green Fluorescent Protein (GFP) detection methodology. All selected extracts and selected essential oils of *A. indica*, *C. javanicum* and *B. retusa* exhibited non-cytotoxic against a primate cell line (Vero cell).

The methanol extract of *A. indica* (aerial parts) was selected for fractionation. Fractionatiom and purification of the active fraction PA21, afforded compound PA21.9* (after recrystallization with methanol), whose structure was elucidated as L-quebrachitol. A reversed phase high performance liquid chromatographic (RP-HPLC) method was also developed for quantitative analysis of L-quebrachitol in *A. indica*.

Isolation and purification of the methanolic extract of *B. retusa* fruits, yielded compound TfM 8.5* (white crystalline powder) whose structure was elucidated as benzoic acid.

Fraction and purification of hexane extract of *C. javanicum* fruits DfH7, afforded compounds DfH7.2*, DfH7.3.2*, DfH7.3.3*, DfH7.3.4*, DfH7.3.5* and DfH7.4* whose structure were elucidated as stigmasterol.

Fractions DfH7.2* and DfH7.3.2* were subjected to GC-MS analysis. The compounds identified from fraction DfH7.2* were 2-pentadecanone, hexadecanoic acid, bis(2-ethylhexyl)phthalate, ergost-5-en-3-ol, (3.beta) and stigmasterol. The constituents identified from fraction DfH7.3.2* were Stigmasta-5, 22-dien-3-ol, (3.beta, 22E) and gamma-sitosterol. Bis(2-ethylhexyl)phthalate is probably contaminant later than an essential oil component. It is a plasticizer contaminant.

Therefore, the extracts and the essential oils of *A. indica*, *C. javanicum* and *B. retusa* showed cytotoxic activity against three human cancer cell lines: KB, MCF7 and NCI-H187 cancer cell lines, but showed non-cytotoxic to vero cells. These medicinal plants also possessed antimicrobial and antioxidant activities. They may play important roles in drug development and as a health supplement and in aromatherapy and food preservation.