

UNIVERSITI TEKNOLOGI MARA

**PHYTOCHEMICAL AND ANTIOXIDANT
STUDIES OF MALAYSIAN MEDICINAL
PLANTS *SYZYGIUM POLYANTHUM* AND
*OCTOMELES SUMATRANA***

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Thesis submitted in fulfilment
of the requirement for the degree of
Doctor of Philosophy

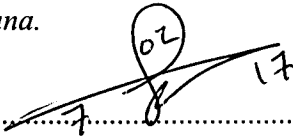
Faculty of Pharmacy

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with Academic Rules and Regulations for post Graduate of Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

The leaves of *S. polyanthum* (Myrtaceae) and barks of *O. sumatrana* (Datisceae) were investigated for their chemical constituents, antioxidant and cytoprotective activities. Their aqueous extracts were first subjected to acidic hydrolysis and the organic layers were dissolved in water and partitioned using hexane, ethyl acetate (EtOAc) and *n*-butanol (BuOH). Six compounds (betulinic acid, ellagic acid, kaempferol, myricetin, quercetin, and β -sitosterol) were isolated and identified from the EtOAc and BuOH extracts of *S. polyanthum* and four compounds (quercetin, kaempferol, rutin, bryonolic acid) were purified from the *n*-butanol extract of *O. sumatrana* by means of MPLC and HPLC. The structures of the above compounds were determined by comparing their NMR and LCMS-TOF data with reported values. The structure of bryonolic acid was further confirmed by X-ray crystallography. Eleven essential oil components (α -caryophyllene, β -caryophyllene, caryophyllene oxide, 1,8-cineole, β -elemene, eugenol, eugenol acetate, isoeugenol, α -pinene, β -pinene, terpinen-4-ol) were identified from *S. polyanthum* and four fatty acid derivatives (linoleic acid, methyl linoleate, myristic acid, palmitic acid) and three steroids [5,6-dihydroergosterol, ergosta-5,8(14)-dien-3 β -ol, ergosta-5-en-3 β -ol] were determined from *O. sumatrana* by GC-MS analysis of their hexane extracts. The *n*-hexane, EtOAc and BuOH extracts were subjected to DPPH, FRAP and cytoprotective activities. The EtOAc and BuOH extracts of both plants showed potent DPPH activity with the EC₅₀ values of $159.12 \pm 0.11 \mu\text{g/mL}$ and $186.40 \pm 0.58 \mu\text{g/mL}$ in *S. polyanthum* and $125.3 \pm 0.17 \mu\text{g/mL}$ and $136.4 \pm 0.17 \mu\text{g/mL}$ in *O. sumatrana*, respectively. It was found that bryonolic acid (EC₅₀ = 26.7 ± 0.74) only marginally quenched DPPH radical but ellagic acid, myricetin, quercetin, rutin and kaempferol (92.4 ± 3.82 , 74.1 ± 1.29 , 76.04 ± 2.63 , 76.8 ± 1.11 and 71.22 ± 1.09 (μM), respectively) showed strong DPPH radical scavenging activity. Then, the isolated compounds from *S. polyanthum* and *O. sumatrana* (myricetin, ellagic acid, betulinic acid, β -sitosterol, rutin, quercetin, kaempferol and bryonolic acid) were tested for their cytotoxic effects towards three types of cells including normal human embryonic liver (WRL-68), normal green monkey kidney (Vero) and human hepatocarcinoma (HepG2) cell lines. The cells were treated with different concentrations of the compounds and the results showed that the compounds from *S. polyanthum* and *O. sumatrana* were non-toxic towards normal cells. However, betulinic acid and bryonolic acid had high cytotoxicity towards HepG2 cells. Next, the cytoprotective effects of the isolated compounds against hydrogen peroxide-induced WRL-68 and Vero cells were investigated. Quercetin, kaempferol, myricetin, ellagic acid, betulinic acid, β -sitosterol and bryonolic acid showed significant protective effects compared to control against oxidative stress-induced WRL-68 and Vero cells. Furthermore, betulinic acid and bryonolic acid showed higher protective effect compared to ellagic acid, kaempferol, myricetin and quercetin and the activities of the antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) were enhanced in a dose-dependent manner. In conclusion, this study demonstrated that most compounds from *S. polyanthum* and *O. sumatrana* were cytoprotective against oxidative stress induced by H₂O₂ with betulinic acid and bryonolic acid having the highest potential to be developed to be used as anticancer candidates and alternative medicine.

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