

## ABSTRACT

Most of the Sarawak's indigenous *Piper* plants are still underutilized and studies on this plant species is a potential and interesting field to be explored. In this research, phytochemical and biological studies have been performed on Sarawak's wild *Piper* spp., namely *P. arborescens*, *P. caninum*, *P. magnibaccum*, *P. aff. longamentum* and *P. erecticaule*. Stem bark and leaves oils of the five *Piper* spp. were extracted using hydro-distillation method. Analysis using Gas Chromatography - Flame Ionization Detector (GC-FID) has identified the presence of various constituents in the essential oil. Three important constituents identified in the stem bark oil of *P. arborescens* were pentadecanal (18.88%), guaiol (11.19%) and  $\beta$ -guaiene (11.12%), whereas important constituents in the stem bark oil of *P. caninum* were identified as isocaryophyllene (20.60%), (*E*)- $\alpha$ -bergamotene (13.74%), (*E*)-isoeugenol (13.46%) and (*E,Z*)-3,6-nonadien-1-ol (9.35%). Analysis on the stem bark oil of *P. magnibaccum* identified two important constituents identified as  $\alpha$ -gurjunene (42.92%) and aromadendrene (24.48%), while  $\alpha$ -gurjunene (29.59%), aromadendrene (15.67%), butyl isothiocyanate (10.71%) and butylmethoxypyrazine (10.51%) were present as important constituents in the leaves oil. Studies on the woody plant of *P. aff. longamentum* and *P. erecticaule* identified pentadecanal as a major constituent in the stem bark and leaves oils with percentage of 79.88% and 81.55%, respectively in *P. aff. longamentum*, and 82.29% and 47.41%, respectively in the stem bark and leaves oil of *P. erecticaule*. Secondary metabolites from the crude extract of *P. arborescens* were isolated and purified by using column chromatography, whereas structural elucidation was performed by using various spectroscopic information especially Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR). Five secondary metabolites have been purified and characterized from the stem bark of *P. arborescens*, which include two terpenes namely caryophyllene oxide and  $\alpha$ -bisabolol,

two alkaloids namely benzamide 2-(methylamino) and piperine, and one phenylpropanoid identified as methyl eugenol. Brine shrimp toxicity assay using *Artemia salina* showed that hexane, dichloromethane, chloroform and methanol crude extracts of *P. arborescens* gave higher cytotoxicity against *Artemia salina* with LC<sub>50</sub> value of 13.12, 16.51, 26.56 and 58.70 µg/mL, respectively. Higher cytotoxicity was also shown by the essential oil of *P. arborescens* with LC<sub>50</sub> value of 57.95 µg/mL. However, essential oil of *P. caninum* showed a significantly lower cytotoxicity with LC<sub>50</sub> value of 249.74 µg/mL. Greater cytotoxicity of the four crude extracts and essential oil of *P. arborescens* indicated the presence of potent cytotoxic components in this *Piper* spp. Antioxidant assay against 2-diphenyl-1-picrylhydrazyl (DPPH) using methanol, dichloromethane, chloroform and hexane crude extracts of *P. arborescens* gave IC<sub>50</sub> value of 21.68, 23.82, 32.88 and 36.54 µg/mL, respectively, which indicated a moderate antioxidant activity. Studies on the essential oils of *P. arborescens*, *P. caninum*, *P. magnibaccum*, *P. aff. longamentum* and *P. erecticaule* however indicated low scavenging activities with IC<sub>50</sub> values of 249.30, 238.70, 211.10, 157.00 and 114.20 µg/mL, respectively. Antibacterial assay of the crude extracts and essential oil of *P. arborescens* against various Gram-negative and Gram-positive bacteria showed weak inhibition except for the hexane crude extract which showed potential antibacterial activity against *Listeria monocytogenes* with inhibition zone of 15.60±1.15 mm.

**Keywords :** *Piper* spp., secondary metabolites, essential oils, antioxidant, toxicity, antibacterial

## ***Minyak Pati Piper spp. serta Kajian Fitokimia dan Aktiviti Biologi Piper arborescens***

### **ABSTRAK**

*Kebanyakan Piper spp. liar di Sarawak masih kurang digunakan dan kajian ke atas tumbuhan ini adalah bidang yang berpotensi dan menarik untuk dilaksanakan. Dalam kajian ini, analisis fitokimia dan biologi telah dilakukan ke atas Piper spp. di Sarawak, iaitu P. arborescens, P. caninum, P. magnibaccum, P. aff. longamentum dan P. erecticaule. Minyak pati daripada lima Piper spp. telah diekstrak dengan menggunakan kaedah penyulingan hidro. Analisis menggunakan Kromatografi Gas - Pengesan Nyalaan Pengionan (GC-FID) telah mengenalpasti tiga komponen penting dalam minyak kulit batang P. arborescens iaitu pentadekanal (18.88%), guaiol (11.19%) dan  $\beta$ -guaiena (11.12%), manakala komponen penting dalam minyak kulit batang P. caninum adalah isokaryofilena (20.60%), (E)- $\alpha$ -bergamotena (13.74%), isoeugenol (13.46%) dan (E,Z)-3,6-nonadien-1-ol (9.35%). Analisis ke atas minyak kulit batang P. magnibaccum telah mengenalpasti dua komponen penting iaitu  $\alpha$ -gurjunena (42.92%) dan aromadendrena (24.48%), manakala  $\alpha$ -gurjunena (29.59%), aromadendrena (15.67%), butil isotiosinat (10.71%) dan butilmetoksipirazina (10.51%) dikenalpasti sebagai komponen penting dalam minyak daun. Kajian ke atas P. aff. longamentum dan P. erecticaule telah mengenalpasti kehadiran pentadekanal sebagai komponen utama dalam minyak kulit batang dan daun dengan jumlah 79.88% dan 81.55% masing-masingnya, pada P. aff. longamentum, serta 82.29% dan 47.41% masing-masingnya, dalam minyak kulit batang dan daun P. erecticaule. Metabolit sekunder tulen daripada Piper arborescens telah diekstrak dan dituliskan dengan menggunakan teknik kromatografi turus dan penentuan struktur telah dilakukan berdasarkan maklumat spektroskopi khususnya Spektrometri Jisim (MS), Resonans Magnetik Nuklear (NMR) dan Inframerah (FTIR).*

*Sebanyak lima metabolit sekunder telah dituliskan dan dicirikan daripada kulit batang P. arborescens, yang menunjukkan kehadiran dua terpena iaitu karyofilena oksida dan  $\alpha$ -bisabolol, dua alkaloid iaitu benzamida 2-(metilamino) dan piperina, serta satu fenilpropanoid iaitu metil eugenol. Ujian ketoksikan terhadap Artemia salina menunjukkan ekstrak heksana, diklorometana, kloroform dan metanol dari P. arborescens memberikan aktiviti sitotoksik yang tinggi terhadap Artemia salina dengan nilai  $LC_{50}$  13.12, 16.51, 26.56 dan 58.70  $\mu\text{g/mL}$ , masing-masingnya. Aktiviti sitotoksik yang tinggi turut ditunjukkan oleh minyak pati P. arborescens dengan nilai  $LC_{50}$  57.95  $\mu\text{g/mL}$ . Walaubagaimapun, minyak pati P. caninum menunjukkan aktiviti sitotoksik yang rendah dengan nilai  $LC_{50}$  249.74  $\mu\text{g/mL}$ . Aktiviti sitotoksik yang tinggi pada ekstrak kasar dan minyak pati P. arborescens menunjukkan kehadiran komponen sitotoksik yang berpotensi dalam Piper spp. Ujian antipengoksidan terhadap 2-difenil-1-pikrilhidrazil (DPPH) menggunakan ekstrak metanol, diklorometana, kloroform dan heksana dari P. arborescens memberikan nilai  $IC_{50}$  21.68, 23.82, 32.88 dan 36.54  $\mu\text{g/mL}$ , masing-masingnya yang menunjukkan ciri antipengoksidan yang sederhana. Kajian ke atas minyak pati P. arborescens, P. caninum, P. magnibaccum, P. aff. longamentum dan P. erecticaule bagaimanapun menunjukkan aktiviti pemerangkapan yang rendah terhadap DPPH dengan nilai  $IC_{50}$  249.30, 238.70, 211.10, 157.00 dan 114.20  $\mu\text{g/mL}$ , masing-masingnya. Ujian antibakteria terhadap ekstrak P. arborescens ke atas beberapa bakteria Gram-negatif dan Gram-positif menunjukkan perencatan yang rendah kecuali bagi ekstrak heksana yang menunjukkan potensi sebagai agen antibakteria terhadap Listeria monocytogenes dengan diameter perencatan  $15.60 \pm 1.15$  mm.*

**Kata kunci :** Piper spp., metabolit sekunder, minyak pati, antioksidan, ketoksikan, antibakteria