

## ABSTRACT

*Phyllagathis rotundifolia* and *P. praetermissa* are herbs from the family of Melastomataceae. They are also known as ‘Tapak Gajah’ or ‘Tapak Sulaiman’ and their extracts are employed as substitute of each other in the traditional medicines. Traditionally, a decoction of the plant is used for fever, malaria, stomachache, parturition and as tonic. However, little is known about their chemical composition and no comparative study on their chemical profiles has been reported. Therefore, the phytochemical analysis would be essential to give an insight into their characteristic chemical components as well as fingerprints that can be used to determine the identity and authenticity of plant material. A total of 22 compounds were isolated from the two species. Among these, seven were identified as galloylated cyanogenic glucosides (**1-7**), 11 were identified as hydrolysable tannins (**8-18**) and two as ellagic acid derivatives (**19, 20**). The remaining two were identified as gallic acid (**21**) and gallic acid methyl ester (**22**). 20 of these compounds were found present in *P. rotundifolia* while nine were found in *P. praetermissa*. The structural identification of these compounds was done using liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Their fragmentation patterns were characterised by tandem mass spectrometry (ESI-MS<sup>n</sup>). Infrared (IR) spectroscopy associated with second derivative spectroscopy and two dimensional infrared (2D-IR) correlation spectroscopy were also employed as a rapid approach for species identification. The cross peak at (1492 cm<sup>-1</sup>, 1181 cm<sup>-1</sup>) was found obvious in the synchronous plot of *P. rotundifolia* but not in *P. praetermissa*. The IR analysis was also used to characterize the *Phyllagathis* species collected from different locations based on their peak positions and intensities. Additionally, a reversed-phase high performance liquid chromatography (HPLC) system was used for the profiling of *P.*

*rotundifolia* and *P. praetermissa* as well as generating the comparative chromatogram for samples from Pasoh Forest Reserve, Ampang Forest Reserve, Johor Forest Reserve and Bukit Lagong. Generally, the galloylated cyanogenic glucosides (peaks **17-22**) were found present in both species except *P. praetermissa* from Bukit Lagong. However, their presence in *P. praetermissa* was relatively lower as compared to *P. rotundifolia*. Similarly, the ellagic acid derivatives 3'-*O*-methyl-3,4-methylenedioxyellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (peak **15**) and 3,3',4-tri-*O*-methylellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (peak **16**) also exhibited lower intensity in the chromatogram of *P. praetermissa* than *P. rotundifolia*. Furthermore, the 1D-IR spectra and HPLC chromatograms were subjected to principal component analysis (PCA) in order to enhance the fingerprinting by converting the huge numerical data to visual plots. The resulting PCA scores plots were able to classify the species into distinct groups which thus can be feasibly applied in quality control measure. The biological potentials of the isolated compounds were evaluated by their neuroprotective activity against H<sub>2</sub>O<sub>2</sub> induced cell damage, *in vitro* cytotoxicity on CaSki (cervical epidermoid carcinoma cells), HCT 116 (colon carcinoma cells), MCF 7 (breast carcinoma) and methicillin-resistant *Staphylococcus aureus* (MRSA) inhibitory activity. Compounds 1,2,3,4,6-penta-*O*-galloyl- $\beta$ -D-glucose (**14**), pterocarinin C (**17**) and prunasin 2',3',4',6'-tetra-*O*-gallate (**7**) highly protected the NG108-15 cell line against H<sub>2</sub>O<sub>2</sub> induction. Both 3'-*O*-methyl-3,4-methylenedioxyellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (**19**) and 3,3',4-tri-*O*-methylellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (**20**) demonstrated low IC<sub>50</sub> values in the CaSki, HCT 116 and MCF 7 carcinoma cell lines. In the case of anti-MRSA activity, prunasin 2',3',4',6'-tetra-*O*-gallate (**7**) displayed the lowest MIC value and thus could be further explored as a potential antimicrobial agent.

## ABSTRAK

*Phyllagathis rotundifolia* dan *P. praetermissa* adalah tumbuhan jenis herba dalam famili Melastomataceae. Kedua-dua spesies juga dikenali sebagai ‘Tapak Gajah’ atau ‘Tapak Sulaiman’ dan ekstraknya sering digunakan sebagai pengganti antara satu sama lain dalam ubatan tradisional. Secara tradisi, rebusan air herba tersebut diguna bagi rawatan demam, malaria, sakit perut serta kelahiran dan sebagai tonik. Walau bagaimanapun, tiada maklumat mengenai komposisi kimia spesies-spesies tersebut dan tiada kajian perbandingan ke atas profil kimia yang dilaporkan. Maka, analisis fitokimia adalah penting bagi pencirian komponen kimia serta pencap-jarian untuk menentukan identiti dan kesahihan identiti tumbuhan. Terdapat sejumlah dua puluh dua sebatian telah diasingkan daripada kedua-dua spesies. Antaranya, tujuh sebatian telah dikenalpasti sebagai galloylated cyanogenic glucosides (**1-7**), sebelas dikenalpasti sebagai hydrolysable tannins (**8-18**) and dua dikenalpasti sebagai derivatif acid ellagic (**19, 20**). Baki dua sebatian telah dikenalpasti sebagai asid galik (**21**) dan metil ester asid galik (**22**). Daripada jumlah ini, dua puluh sebatian didapati hadir dalam *P. rotundifolia* manakala sembilan ditemui di dalam *P. praetermissa*. Penentuan struktur sebatian tersebut adalah berdasarkan kromatografi cecair-spektrometri jisim (LC-MS) dan spektroskopi resonans magnetik nuklear (NMR). Corak pemecahan bagi sebatian pula dicirikan dengan spektrometri jisim tandem (ESI-MS<sup>n</sup>). Spektroskopi inframerah (IR) yang merangkumi spektroskopi terbitan kedua serta spektroskopi inframerah korelasi dua dimensi (2D-IR) telah digunakan sebagai satu pendekatan untuk pengenalpastian identiti spesies. Puncak rentas pada (1492 cm<sup>-1</sup>, 1181 cm<sup>-1</sup>) yang didapati dalam plot synchronous adalah jelas bagi *P. rotundifolia* tetapi tidak jelas bagi *P. praetermissa*. IR spektroskopi boleh juga digunakan untuk mencirikan *Phyllagathis* spesies yang dikutip dari lokasi yang berlainan berdasarkan kedudukan dan

keamatan puncak. Selain daripada itu, fasa terbalik kromatografi cecair berprestasi tinggi (HPLC) juga digunakan untuk pemprofilan *P. rotundifolia* dan *P. praetermissa* serta membuat perbandingan antara tumbuhan dari Hutan Simpanan Pasoh, Hutan Simpanan Ampang, Hutan Simpanan Johor dan Bukit Lagong. Secara umum, galloylated cyanogenic glucosides (puncak **17-22**) didapati hadir dalam kedua-dua spesies kecuali *P. Praetermissa* dari Bukit Lagong. Walau bagaimanapun, kehadiran mereka di *P. praetermissa* adalah lebih rendah secara relatif berbanding dengan *P. rotunfolia*. Bersama dengan kompaun 3'-*O*-methyl-3,4-methylenedioxyellagic 4'-*O*- $\beta$ -D-glucopyranoside (puncak **15**) dan 3,3',4-tri-*O*-methylellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (puncak **16**) juga menunjukkan intensiti yang lebih rendah bagi kromatogram *P. praetermissa* berbanding dengan *P. rotundifolia*. Seterusnya, spektrum 1D-IR dan HPLC kromatogram juga dianalisis dengan menggunakan principal component analysis (PCA). Analisis ini dapat meningkatkan potensi pencap-jarian daripada menukar maklumat berangka yang besar kepada plot visual. PC scores plot yang dijana dapat mengklasifikasikan spesies kepada kumpulan yang berbeza dan dapat dilaksanakan bagi kawalan kualiti. Potensi biologi bagi sebatian-sebatian tersebut juga dinilai untuk aktiviti neuroprotective, *in vitro* sitotoksik CaSki (karsinoma serviks epidermoid), HCT 116 (karsinoma kolon), MCF 7 (karsinoma payudara) dan aktiviti anti-methicillin resistant *Staphylococcus aureus* (MRSA) Sebatian 1,2,3,4,6-penta-*O*-galloyl- $\beta$ -D-glucose (**14**), pterocarinin C (**17**) dan prunasin 2',3',4',6'-tetra-*O*-gallate (**7**) didapati melindungi sel NG108-15 neuroblastoma-glioma hybrid terhadap induksi H<sub>2</sub>O<sub>2</sub>. Kedua-dua sebatian 3'-*O*-methyl-3,4-methylenedioxyellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (**19**) dan 3,3',4-tri-*O*-methylellagic acid 4'-*O*- $\beta$ -D-glucopyranoside (**20**) menunjukkan nilai IC<sub>50</sub> yang rendah dalam CaSki, HCT 116 dan MCF 7 sel karsinoma. Manakala bagi aktiviti anti-MRSA, prunasin 2',3',4',6'-tetra-*O*-gallate (**7**) menunjukkan nilai MIC yang terendah dan boleh diterokai sebagai ejen anti-mikrob yang berpotensi.