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ORTHOGONAL CHROMATOGRAPHY FOR THE ISOLATION AND PURIFICATION OF MINOR BIOACTIVE METABOLITES FROM A MALAYSIAN ENDOPHYTIC FUNGI

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ABSTRACT

Endophytic *Aspergillus* HAB10R12 was isolated from *Garcinia Scortechinii* (Kenondong Krot @ Kandis), collected in a secondary forest in Kuala Pilah. It was shown to contain notably 5 cytotoxic peptides, for which a patent was applied. In order to further develop these compounds, larger quantities were separated. Unfortunately, these peptides are produced in a very small quantity together with a number of non active compounds. For this purpose (100 20-cm Petri dishes) were inoculated and fermented according to a standardized procedure, by batches of 10 plates each. Each batch was extracted based on our standard operation procedure. Each extract was then subjected to LC/MS/MS technology Agilent 1200 series system equipped with Agilent 6410 Triple Quad. Total ion chromatography (TIC) was done and followed by ion collusion technology to produce (Product ion) and (precursor ion) to prove the presence of these peptides in the extract. In this process, additional peptides were detected. The purification of those peptides was carried out using two successive orthogonal separation steps. The 1st one is a gel filtration process using Preparative HPLC from Japan analytical industry using Jaigael H column (Polymeric GPC column i.d. 20 X 600mm) medium pressure, using Chloroform and Tetrahydrofuran as a solvent system with 2.5 mL/min flow rate. The presence of the expected compounds in one of the fractions was confirmed by LC/MS/MS. The peptides were then fractionated onto an analytical column in HPLC Agilent 1200 series system equipped with a diode-array detector (DAD) and evaporative light scattering detector (ELSD). All analyses and separations were carried out in a reverse phase mode, using a Synergy 4u Hydro-RP 80Å column (150 × 4.6 mm, 4 µm particle size, Phenomenex®, USA with flow rate 1 mL/min) with a guard column filled with the same material. The column temperature was maintained at 36°C. The monitoring of the separation is carried out by both a DAD and an ELSD (as these peptides are not much UV-active). A fraction collector connected to the LC system allowed the collection of the pure targeted peptides. Their identity was confirmed by LC-MS[2] and capillary NMR.