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Isolation of Clitorin and Manghaslin from Carica papaya L. Leaves by CPC and Its Quantitative Analysis by QNMR

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Abstract : Papaya (Carica papaya L., Caricaceae) is a tree which mainly cultivated for its fruits in many tropical regions including Australia, Brazil, China, Hawaii, and Malaysia. Beside of fruits, its leaves, seeds, and latex have also been traditionally used for treating diseases, which also reported to possess anti-cancer and anti- malaria properties. Its leaves have been reported to consist of various chemical compounds such as alkaloids, flavonoids and phenolics. Clitorin and manghaslin are among major flavonoids presence. Thus, the aim of this study is to quantify the purity of these isolated compounds (clitorin and manghsalin) by using quantitative Nuclear Magnetic Resonance (qNMR) analysis. Only fresh C. papaya leaves were used for juice extraction procedure and subsequently was freeze-dried to obtain a dark green powdered form of the extract prior to Centrifugal Partition Chromatography (CPC) separation. The CPC experiments were performed using a two-phase solvent system comprising ethyl acetate/butanol/water (1:4:5, v/v/v/v) solvent. The upper organic phase was used as the stationary phase, and the lower aqueous phase was employed as the mobile phase. Ten fractions were obtained after an hour runtime analysis. Fraction 6 and fraction 8 has been identified as clitorin (m/z 739.21 [M-H]-) and manghaslin (m/z 755.21 [M-H]-), respectively, based on LCMS data and full analysis of NMR (1H NMR, 13C NMR, HMBC, and HSQC). The 1H-qNMR measurements were carried out using a 400 MHz NMR spectrometer (JEOL ECS 400MHz, Japan) and deuterated methanol was used as a solvent. Quantification was performed using the AQARI method (Accurate Quantitative NMR) with deuterated 1,4-Bis(trimethylsilyl)benzene (BTMSB) as an internal reference substances. This AQARI protocol includes not only NMR measurement but also sample preparation that provide highest precision and accuracy than other qNMR methods. The 90° pulse length and the T1 relaxation times for compounds and BTMSB were determined prior to the quantification to give the best signal-to-noise ratio. Regions containing the two downfield signals from aromatic part (6.00–6.89 ppm), and the singlet signal, (18H) arising from BTMSB (0.63-1.05ppm) were selected for integration. The purity of clitorin and manghaslin were calculated to be 52.22% and 43.36%, respectively. Further purification is needed in order to increase its purity. This finding has demonstrated the use of qNMR for quality control and standardization of various plant extracts and which can be applied for NMR fingerprinting of other plant-based products with good reproducibility and in the case where commercial standards is not readily available.

Keywords : Carica papaya, clitorin, manghaslin, quantitative Nuclear Magnetic Resonance, Centrifugal Partition Chromatography
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