UNIVERSITI TEKNOLOGI MARA

QUANTIFICATION OF FLAVONOIDS FROM MALAYSIAN *UNCARIA* SP. USING HPLC-DAD

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of the requirements for the degree of
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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on to conduct the final examination of Nurliayana Binti Ibrahim on her Master of Science thesis entitled "Quantification of Flavonoids from Malaysian *Uncaria* Sp. Using HPLC-DAD" in accordance with Universiti Teknologi MARA Act 76 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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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 results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

Phytochemical investigation on the methanolic extract of the leaves and stems of Malaysian *Uncaria* plants was carried out by developing, optimizing and validating an HPLC method for the detection and identification of flavonoids in Malaysian *Uncaria gambir* using analytical HPLC-DAD and TOF-LCMS. The HPLC method was successfully developed with the mobile phase composition of mobile phase A (0.1 % TFA in 5 % ACN) and mobile phase B (0.1 % TFA in ACN) at a flowrate of 0.8 mL/min. The gradient elution of the mobile phases were 0-10 min: 88-82 % A; 10-15 min: 82 % A (isocratic); 15-30 min: 50 % A. The method was validated according to ICH guidelines in terms of linearity, accuracy and precision and robustness. Linearity was observed with coefficient of correlation, $r^2 \geq 0.9951$ for all the investigated analytes. The percentage recovery was found within the range of 90.07 - 106.52 %. The percentage relative standard deviation for accuracy and precision was discovered to be less than 2 %. The developed method was proven to be an efficient, selective and rapid analytical method which allowed simultaneous detection of the flavonoids. The flavonoids of interest were then isolated from the leaf extract of *U.gambir*-2 via a scale-up method employing preparative HPLC-DAD. Characterization of the isolated flavonoids were based on spectroscopic evidence and comparison with literature values. A total of four flavonoids comprising two flavan-3-ols (catechin, UGL2-1 and *epi*catechin, UGL2-2) and two flavonols (rutin, UGL2-3 and quercetin, UGL2-4) with mass of 12.2 mg, 6.43 mg, 6.02 mg and 6.67 mg, respectively were successfully isolated and characterized. The four isolated flavonoids including *epi*afzelechin (previously isolated from *U. longiflora* var *pteropoda)* were then used as standards for the quantification analysis of twenty-two samples comprising six Malaysian *Uncaria* species via analytical HPLC-DAD. The amount of catechin (analyte 1) and quercetin (analyte 5) were found highest in the leaf extract of *U. gambir*-2, UG2 with 194.6 mg/g and 36.77 mg/g, respectively. *Epi*catechin (analyte 2) was found highest in the leaf extract of *Uncaria acida*-2 (UA2) with 30.62 mg/g meanwhile, rutin (analyte 4) was found highest in leaf extract of *Uncaria lanosa* var. *ferrea* (ULAF) with 94.91 mg/g. *Epi*afzelechin (analyte 3) was only detected in *U. longiflora* species with the highest amount in the stem extract of ULO2 with 35.67 mg/g.

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