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Metabolomic Analysis of *Eurycoma Longifolia* JACK Roots by ¹H-NMR Spectroscopy

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The Nuclear Magnetic Resonance (NMR)-based plant metabolomic method was used for the analysis of 29 *Eurycoma longifolia* Jack (Tongkat Ali) roots, harvested from the same source in Perak. The objective of study was to establish a standardised profile of the plant metabolites for comparison with those of *E. longifolia* root samples derived from other sources. The age of the plant, the climatic conditions, soil pH and geographical locations were considered as probable variables. The roots were extracted with distilled-diionized water. The ¹H-NMR profile of each extract and the major secondary quassinoid metabolites, eurycomanone, eurycomanol, and eurycomanol-2-*O*-β-D-glycopyranoside were obtained. The NMR spectra were binned to 0.04 ppm and the data were analysed using the AMIX-TOOLS and SIMCA-P. Preliminary identification of the secondary metabolites confirmed the presence of the quassinoids of study in all the extracts. The Principle Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were performed. The PCA results at 95 % confidence level showed that no outliers were observed among the samples, indicating that the plants were similar in NMR profile. However, from OPLS-DA, five groups were identified to be different in terms of age and were statistically significant at 95 % confidence level. The differences among the groups strongly reflected the changes in the type and level of the metabolites. The identification and quantification of the discriminating metabolites among the groups will be discussed. Further studies on the profiling of the root organic extract and the biomarker quantification are ongoing.

Keywords: Eurycoma longifolia Jack, Quassinoids, NMR metabolomics, OPLS-DA, PCA.