A GEL-BASED PROTEOMIC KIT TO SCREEN THE QUALITY OF WATER-SOLUBLE ROOT EXTRACTS OF *EURYCOMA LONGIFOLIA*

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Eurycoma longifolia, belonging to the family Simaroubaceae, is a small forest tree found in Southeast Asian rain forest areas ranging from Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia and Indonesia (Osman *et al.* 2003). Locally known as tongkat ali in Malaysia, the root of the tree is popular as a traditional medication, in particular as an aphrodisiac. Recently, Ang *et al.* (2003) reported that the root of *E. longifolia* is able to enhance sexual motivation in rats. The root has also been shown to have antimalarial, cytotoxic, antiulcer, antipyretic and aphrodisiac properties (e.g. Ang & Sim 1997, Kuo *et al.* 2004, MMBPP 2005).

Based on the belief that *E. longifolia* root has aphrodisiac properties, its use and consumption has increased in Malaysia and neighbouring countries. Many food products, e.g. coffee and tonic drink, now contain the root as one of their constituents. With greater demand for the product, the price of root extracts available in the market is on the increase. However, root extracts vary in quality as no reliable quality control is currently available.

The purity of *E. longifolia* extract in a given commercial product can be determined through proteomic studies vis-à-vis two-dimensional gel electrophoresis (2DE). This technique involves the separation of proteins that reflect the quality of *E. longifolia* present in a product. To date, scientists face problems in producing protein maps of this species as separating and dissolving its water-soluble root extracts have been unsuccessful due to poor extraction and solubilization of the proteins.

Therefore, there is a need for a standard that is reliable, easy to perform and scientifically valid for determining the quality of root extracts available in the market. To address such a need, the Forest Research Institute Malaysia (FRIM) has developed a standard protein map of water-soluble root extracts of *E. longifolia* using the 2DE technique.

The protocol for the separation was established using a one-stop proteomic kit developed earlier by FRIM. The kit comprises: (1) complete 2DE solution, (2) silver staining solution, and (3) in-gel trypsin digestion solution. All the solutions are compatible with MALDI-MS.

One milligram of water-soluble *E. longifolia* root extract was mixed with sample buffer followed by rehydration solution. After passive rehydration, the first dimension was performed to separate proteins based on isoelectric point (pI). Then the second dimension was performed to separate proteins based on molecular weight. To obtain the protein map, the second dimension gel was stained using silver solution. The protein map showed high consistency and reproducibility with good visibility of protein spots.

With the development of this protocol using the one-stop proteomic kit, producers of tongkat ali products will be able to determine the quality of extracts purchased from their suppliers. More importantly, the producers can also verify that the *E. longifolia* extracts meet their specifications in terms of purity. FRIM has the facilities and expertise to perform these tests for clients requiring such services.

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