

**PROTEOMIC CHARACTERIZATION OF *EURYCOMA
LONGIFOLIA* AND ITS APPLICATION IN VALIDATING
TONGKAT ALI HERBAL PRODUCTCS**

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ABSTRACT

Eurycoma longifolia commonly known as “Tongkat Ali” in Malaysia is claimed to have aphrodisiac properties and has been widely used in Malay traditional medicine. An aphrodisiac is defined as a substance that increases sexual activity and libido and/or improves sexual performance. These claims have led to a vast number of “Tongkat Ali” products being marketed without proper authentication. There was therefore a need to characterize the proteins in these commercial Tongkat Ali herbal products using biomarkers to establish the authenticity of the claims. The important chemical components in *Eurycoma longifolia* are eurycomanol, eurycomanone and eurycomalactone. However they are not linked to the aphrodisiac properties, instead suspected to be protein base. In this study a total of 23 Ministry Of Health (MOH) approved and 6 non-MOH approved samples were sourced and screened for markers previously developed using two dimensional electrophoresis (2-DE). The markers were denoted as A and B with appearances between 12 to 14 kDa in 2-DE performed on IPG strips of 3-10 (7 cm) for the 1st dimensional electrophoresis, while 12.5% homogenous gel was used for the 2nd dimensional electrophoresis in silver stain. The results of the study confirmed that 18 of the 29 products tested were authentic with the presence of both biomarkers A and B, while 11 (a combination of both approved and non-MOH approved products) did not show presence of biomarkers. This enabled authentication of the products tested. The protein markers in addition to the existing chemical marker, Eurycomanone, may serve to be useful in authenticating genuine Tongkat Ali products.

Keywords: *Eurycoma longifolia*, aphrodisiac, protein-biomarker, authentication, Tongkat Ali

ABSTRAK

Eurycoma longifolia yang dikenali secara am sebagai Tongkat Ali di Malaysia dikatakan mempunyai kebolehan membantu lelaki yang menghadapi masalah syahwat. Tumbuhan ini digunakan secara berleluasa di kalangan pengamal perubatan tradisional Melayu. Afrodisiak atau juga dikenali sebagai penggalak seks adalah bahan yang dipercayai boleh meningkatkan keupayaan seks. Fakta ini telah menyebabkan pengeluaran produk yang mengandungi Tongkat Ali dilakukan secara besar-besaran tanpa pengesahan yang sepatutnya. Oleh yang demikian, keperluan untuk menganalisis kandungan protein dalam produk komersial Tongkat Ali menggunakan bio-petanda adalah penting untuk mengesahkan produk-produk tersebut. Bahan kimia yang penting dalam *Eurycoma longifolia* adalah “eurycomanol”, “eurycomanone” dan “eurycomalactone”. Walaubagaimanapun, bahan-bahan kimia tersebut dikatakan tidak mempunyai peranan ke atas unsur afrodisiak yang dinyatakan. Sebaliknya, unsur afrodisiak dalam Tongkat Ali dikaitkan dengan kandungan protein dalam tumbuhan tersebut. Merujuk kepada fakta tersebut, dalam kajian ini, 23 produk Tongkat Ali yang diiktiraf oleh Kementerian Kesihatan dan 6 produk Tongkat Ali yang tidak mempunyai pengesahan oleh Kementerian Kesihatan dipilih dan disaring sama ada produk-produk tersebut mempunyai protein-protein bio-penanda Tongkat Ali. Protein-protein bio-penanda tersebut dirujuk sebagai A dan B dengan jisim molekul seberat antara 12 ke 14 kDa melalui gel elektroforesis dua dimensi (2-DE) yang menggunakan jalur IPG pH 3-10 (7 cm) untuk kaedah dimensi pertama (pemfokusan isoelektrik), manakala 12.5% gel homogenus digunakan dalam kaedah dimensi kedua diwarnakan dengan pewarna perak (silver stain). Keputusan kajian mengesahkan bahawa 18 daripada 29 produk yang dikaji mengandungi kedua-dua penanda-bio A dan B, manakala 11 (kombinasi

daripada 23 produk yang diiktiraf dan 6 produk yang tidak diiktiraf oleh Kementerian Kesihatan) tidak mengandungi kedua-dua penanda-bio A dan B. Kesimpulannya, kaedah analisis secara elektroforesis dua dimensi dengan penanda-bio membantu dalam pengesahan produk Tongkat Ali. Penanda-bio protein sebagai tambahan kepada penanda-kimia yang telah lama digunakan, Eurycomanone, akan membantu dalam proses pengesahan produk-produk Tongkat Ali yang bermutu.

Kata kunci: *Eurycoma longifolia*, afrodisiak, protein penanda-bio, pengesahan, Tongkat Ali.

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LIST OF ABBREVIATIONS

2-DE	2-dimensional electrophoresis
APS	Ammonium persulfate
BSA	Bovine serum albumin
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
ddH ₂ O	Distilled deionized water
DTT	Dithiothreitol
HCl	Hydrochloric acid
hrs	Hours
IEF	Isoelectric Focusing
M	Molar
Min	Minute
mM	Milimolar
mg	Miligram
MOH	Ministry of Health
pH	Negative logarithm of hydrogen ion concentration
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis
TEMED	Tetramethylethylenediamine
w/v	Weight per volume
μl	Microliter