CHARACTERIZING AND ISOLATING PROTEIN MARKER IN AUTHENTICATING *EURYCOMA LONGIFOLIA* HERBAL PRODUCTS

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ABSTRACT

Eurycoma longifolia or commonly known as Tongkat Ali has been identified as a valuable product in phytochemical industry due to its reputation in enhancing sexual properties. The proliferation of *E. longifolia* based herbal products renders quality control measure to be an important task. Standardization should be carried out, and currently the products are standardised to eurycomanone, the primary compound in the plant. Current research is on preliminary work in developing and isolating protein as marker compound to authenticate *E. longifolia* herbal products. From the market, 16 Malaysian Registered Products, 14 Malaysian Unregistered Products, 12 International Products and 8 Beverages were sampled. Eurycomanone analysis revealed that 24 or 48% of the total products contained eurycomanone while 26 or 52% did not. Protein marker analysis was commenced with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE). The results indicated that a distinctive single protein band appeared in the SDS PAGE gel of product containing *E. longifolia*. Further inspection by 2 dimensional gel electrophoresis (2DE) revealed that *E. longifolia* consisted of four proteins, with similar molecular weight, but differed by isoelectric point. The four proteins were denoted as Marker A, B, C and D. Marker A was chosen as the ultimate marker as it was consistently presented in products containing *E. longifolia*. The presence and quantity of eurycomanone and Marker A in products containing *E. longifolia* was comparable with minor exception for four products (C1, C4, C7, and C21). Marker A was isolated using subsequent size exclusion chromatography and anion exchange chromatography. The purity of Marker A was proven by the appearance of single spot in 2DE gel, with the same electrophoretic profile of Marker A in *E. longifolia* extracts. Marker A then characterized by MALDI TOF MS and partially sequenced using *de novo* sequencing method. Marker A consisted of 22 amino acids. This study has led to the isolation of homogenous protein that can be utilized as novel and comparable marker to the chemical marker; eurycomanone, to authenticate *E. longifolia* products. Being a protein, subsequently an antibody can be developed and incorporated into biosensor device.

ABSTRAK

Eurycoma longifolia atau lebih dikenali sebagai Tongkat Ali adalah satu produk yang bernilai di dalam industri fitokimia disebabkan reputasinya untuk meningkatkan fungsi seksual. Peningkatan produk *E. longifolia* di pasaran menjadikan kawalan kualiti sebagai sesuatu yang penting, tambahan pula timbul isu produk ini dipalsukan. Piawaian mesti dijalankan dan pada masa ini, ia dipiawaikan kepada eurycomanone, bahan utama di dalam *E. longifolia*. Kajian ini adalah berkenaan menjadikan *protein* sebagai penanda kepada produk *E. longifolia*. 16 Produk Malaysia Berdaftar, 14 Produk Malaysia Tidak Berdaftar, 12 Produk Antarabangsa, 8 Produk Minuman telah disampel dari pasaran. Analisa *eurycomanone* menunjukkan 24 atau 48% dari keseluruhan produk mengandungi *eurycomanone* manakala 26 atau 52% tidak mengandungi *eurycomanone*. Analisa *protein* penanda dimulakan dengan *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis* (SDS PAGE). Hasil analisa mendedahkan satu jalur *protein* di dalam gel produk yang mengandungi *E. longifolia*. Analisa lanjutan untuk *protein* penanda dijalankan dengan gel electrophoresis dua dimensi (2DE) mendedahkan *E. longifolia* terdiri dari 4 *protein*, dengan berat molekul yang sama tetapi berbeza takat isoelectrik. 4 *protein* tersebut dinamakan Penanda A, B, C dan D. Penanda A di pilih sebagai penanda terbaik kerana sentiasa hadir di dalam produk yang mengandungi *E. longifolia*. Kehadiran dan kuantiti *eurycomanone* dan Penanda A di dalam produk yang mengandungi *E. longifolia* didapati setara kecuali untuk 4 produk (C1, C4, C7, C21. Penanda A berjaya dipencil menggunakan kromatografi berasas saiz dan kromatografi penukaran ion. Ketulenan Penanda A dibuktikan dengan penampakan satu titik *protein* di dalam gel 2DE dengan sifat elektroforetik yang sama dengan Penanda A. Penanda A dikenal pasti menggunakan MALDI TOF MS dan penjujukan dilakukan menggunakan kaedah penjujukan *de novo*. Hasil penjujukan menunjukkan Penanda A terdiri daripada 22 asid amino, tetapi penjujukan ini adalah separa. Kesimpulannya, kajian ini telah berhasil untuk memencilkan *protein* yang berpotensi untuk menjadi penanda kepada produk *E. longifolia*. Penanda *protein* mempunyai potensi untuk menghasilkan antibodi dan diaplikasi di dalam alat biosensor.