THREE TONGKAT ALI PLANTS *(EURYCOMA LONGIFOLIA*, *POLYALTHIA BULLATA*, AND *STEMA TUBEROSA*) STUDIED FOR THEIR APHRODISIAC POTENTIALS WITH EMPHASIS ON THEIR TESTOSTERONE BOOSTING CAPABILITIES

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MASTER OF SCIENCE

UNIVERSITI MALAYSIA PAHANG

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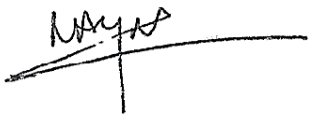

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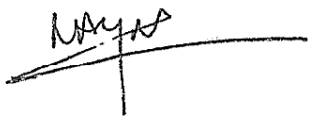

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ABSTRAK

Eurycoma longifolia (EL), *Pollyathia bullata* (PB), dan *Stema tuberosa* (ST) adalah tiga spesies tumbuhan yang mempunyai sinonim "Tongkat Ali" iaitu Tongkat Ali Putih, Tongkat Ali Hitam dan Tongkat Ali Merah, masing-masing. Kemungkinan mereka semua dikenali sebagai Tongkat Ali kerana dipercayai mempunyai keupayaan afrodisiak dan kesemua menggunakan akar sebagai penawar. Dalam kajian ini, ketiga-tiga tumbuhan Tongkat Ali telah diuji dari segi keselamatan dan keberkesanannya sama ada secara *in vivo* (dalam badan) mahupun *in vitro* (luar badan). Tongkat Ali telah dijadikan dalam bentuk kapsul dan diberikan kepada unggas. Sebelum diuji pada haiwan, kandungan kapsul dianalisis dengan beberapa ujian keselamatan dan kualiti, seperti ujian pH dan kelembapan, kandungan logam berat, ujian mikrobiologi dan kehadiran steroid sintetik. Sejumlah, 12 mg setiap serbuk Tongkat Ali dalam bentuk kapsul diberikan kepada ayam selama 30 hari. Hanya untuk PB, rawatan dilanjutkan hingga 60 hari setelah didapati 30 hari tidak berkesan. Hasil kajian menunjukkan peningkatan hormon kelakian testosteron pada ayam dengan kenaikan tertinggi 9.73 ± 1.20 nmol/L oleh PB diikuti oleh EL dan ST, 7.70 ± 0.59 nmol/L dan 6.25 ± 0.70 nmol/L, masing-masing. Komponen protein tumbuhan ini sudah lama disyaki sebagai penyebab ciri-ciri afrodisiak terumtama bagi EL. Oleh itu, kajian ini juga dilakukan untuk mengasingkan dan mencirikan protein dalam spesies Tongkat Ali dengan pencirian dilakukan dengan mengguna ujian penentukuran Bradford dan juga SDS PAGE. Kandungan protein tertinggi adalah pada EL iaitu 14.31% diikuti PB dan ST, masing-masing 7.9% dan 6.51%. Setelah terbukti mengandungi protin dalam ketiga-tiga spesies Tongkat Ali, salah satu jenis tumbuhan Tongkat Ali dipilih bagi aktiviti seterusnya. Protin tumbuhan Tongkat Ali yang lebih popular iaitu EL diasingkan pada mulanya menggunakan kromatografi pengasingan berpandukan saiz mokekular. Seterusnya, diperhalusi lagi dengan kromatografi afiniti terjerap dengan bahan aktif Lectin untuk mendapatkan glikoprotein menggunakan larutan yang boleh menyah-jerap iaitu penampan elusi mannose sebanyak 50%. Hasil fraksi kedua-dua kromatografi dan ekstrak-ekstrak iaitu EL, glikoprotein (fraksi dalam EL), PB dan ST diuji secara *in vitro* menggunakan sel TM-3 Leydig dengan berkepekatan 50 μg/mL. Akibatnya, *E. longifolia* didapati dengan peningkatan perembesan testosteron tertinggi sebanyak 0.26 ng/mL diikuti oleh *P. bullata* 0.17 ng/mL, EL glikoprotein 0.12 ng/mL dan *S. tuberosa* 0.07 ng/mL. Secara ringkas, EL, glikoprotein, PB dan ST disahkan mempunyai kesan dalam meningkatkan tahap testosteron dalam sel TM-3 Leydig. Lebih penting lagi hasilnya juga menunjukkan EL glikoprotein, iaitu bahagian EL yang dapat diasingkan secara mudah dan spesifik menggunakan kromatografi afiniti didapati memberikan kemampuan meningkatkan testosteron, hormon kelakian. Kajian awal ini memberikan bukti dalam kedua-dua ujian *in vitro* dan *in vivo*, ketiga-tiga tumbuhan Tongkat Ali yang dikaji berkeupayaan meningkatkan testosteron dan dengan demikian terbukti mempunyai kesan afrodisiak.

ABSTRACT

Eurycoma longifolia (EL), *Pollyathia bullata* (PB), and *Stema tuberosa* (ST) are three species of plants sharing the synonym of "Tongkat Ali" and commonly known as Tongkat Ali Putih, Tongkat Ali Hitam, and Tongkat Ali Merah, respectively. Perhaps they are all known as Tongkat Ali due to their aphrodisiac claims, and parts used are their roots. In this study, three Tongkat Ali plants were tested for safety and efficacy *in vivo* and *in vitro.* The roots of the plants were encapsulated and given to fowls. Before being tested on animals, the capsules' content was analysed on a few safety and quality parameters, i.e., pH and moisture tests, heavy metal content, microbial load, and steroid presence. Next, 12 mg of each Tongkat Ali powder included in a capsule was given to fowls for 30 days. The treatment was further extended to 60 days; once found, 30 days was not effective. The outcome showed an increase in testosterone in fowls with the highest value of 9.73 ± 1.20 nmol/L obtained by PB, followed by EL and ST, 7.70 ± 0.59 nmol/L, and 6.25 ± 0.70 nmol/L, respectively. The protein components of these plants were believed to be giving rise to aphrodisiac capabilities. Thus, this study has isolated and characterized the Tongkat Ali species' protein by Bradford assay and SDS PAGE. The highest protein content was EL, i.e., 14.31% ± 3.25, followed by PB and ST, 7.9% ± 2.21 and 6.51% ± 2.03, respectively. The protein of the more popular Tongkat Ali plants, i.e., EL, was removed from the organic constituents with smaller molecular weights using size exclusion chromatography. Next, the protein fraction was further refined by Lectin column chromatography to obtain glycoprotein using a 50% mannose elution buffer. Consequently, the EL, EL glycoprotein, PB, and ST were tested *in vitro* using TM-3 Leydig cells with a 50 μg/mL concentration. *E. longifolia* was found to have the highest increase of testosterone secretion of 0.26 ng/mL. This was followed by *P. bullata* of 0.17 ng/mL, EL glycoprotein of 0.12 ng/mL, and *S. tuberosa* of 0.07 ng/mL. The outcome showed the aqueos crude extract of EL, EL glycoprotein, PB, and ST confirmed the effects in alleviating the testosterone level in TM-3 Leydig cells. More importantly, the results have proven EL glycoprotein, a pure constituent of EL, can be easily isolated using affinity chromatography to boost testosterone-boosting capabilities. This preliminary study also has provided *in vitro* and *in vivo* evidence of testosterone boosting capabilities of the three Tongkat Ali plants, proving that they are all, in fact, aphrodisiac as claimed.

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