ACCELERATED AQUEOUS EXTRACTION AND PHYTOCHEMICALS SCREENING OF *EURYCOMA LONGIFOLIA* (TONGKAT ALI) EXTRACT

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Dedicated to my beloved parents, brothers and my sisters, who had provided me with the support spiritually and emotionally throughout the long journey. To my dearest husband; Mat Salleh Yamin and my children; Muhammad Adam Haziq and Nur Aleysha Qurratu'aini, who had motivated me to complete the study.

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ABSTRACT

The development of a rapid, robust and reliable method for extraction of plant materials is important for the screening of a wide range of plant bioactives and the discovery of biomarker. Accelerated aqueous extraction or commercially known as Accelerated Solvent Extraction (ASE) is an automated extraction technique operated at elevated temperatures and pressures to achieve extraction in a short period of time. The high temperature weakens the solute-matrix interactions and leads to a faster diffusion rate, better analyte solubility and lower solvent viscosity. This research was undertaken to evaluate the performance of an accelerated aqueous extraction of eurycomanone and other bioactive compounds from Tongkat Ali. Investigation was carried out to elucidate the effect of static cycle, static time and temperature on the content and degradation of eurycomanone. To date, there is no study being carried out on optimization of the extraction of eurycomanone from Tongkat Ali roots using this technique. The optimum operating conditions were subsequently used for the extraction of other phytochemicals. Response surface methodology was used to determine the significant operating conditions. The Box-Behnken design was implemented to maximize the response (eurycomanone content) from the resulted response surface. The extraction yield of eurycomanone are mainly affected by temperature (>100 ^{o}C) followed by the static time. A higher static time (>11 min) was found to cause eurycomanone degradation, while a lower temperature and static time reduced the extraction efficiency. The optimum conditions yielded a corresponding eurycomanone content of 9.21mg/g at static time of 8 minutes, static cycle of 5 and temperature of 90 ^{o}C. A liquid chromatography coupled with a triple quadrapole and time-of-flight, mass spectrometer (LC-QTOF-MS/MS) was used to profile the small metabolites. The major quassinoid identified were 13α(21)-epoxyeurycomanone, eurycomanone, longilactone14, 15β-dihydroxyklaineanone, 6α-hydroxyeurycomalactone, eurycomalide B, laurycolactone A and laurycolactone B. In summary, the combination method of ASE and statistical analysis presented is an expedient technique for the phytochemicals screening of Tongkat Ali roots.

ABSTRAK

Pembangunan kaedah yang cepat, tahan lasak dan diyakini untuk mengekstrak tumbuhan adalah penting untuk menyaring pelbagai bioaktif dan penemuan penanda-bio sesuatu tumbuhan. *Accelerated Aqueous Extraction* atau secara komersial dikenali sebagai *Accelerated Solvent Extraction* (ASE) adalah teknik pengekstrakan automatik beroperasi pada suhu dan tekanan tinggi untuk mencapai pengekstrakan dalam tempoh yang singkat. Suhu yang tinggi melemahkan interaksi antara bahan larut-matriks dan menghasilkan kadar resapan yang cepat, analit melarut dengan lebih baik dan kelikatan pelarut yang rendah. Kajian ini dijalankan untuk menilai prestasi kaedah *accelerated aqueous extraction* untuk *eurycomanone* dan sebation bioaktif lain daripada Tongkat Ali. Kajian telah dijalankan bagi menjelaskan kesan pengaruh kitaran statik, masa statik dan suhu terhadap kandungan serta degradasi *eurycomanone*. Sehingga kini, tiada kajian dijalankan untuk mengoptimumkan pengestrakan *eurycomanone* dari Tongkat Ali menggunakan kaedah ini. Keadaan operasi yang optimum kemudiannya digunakan untuk mengekstrak fitokimia lain. Perisian *response surface methodology* (RSM) digunakan untuk menentukan keadaan operasi yang ketara. Reka bentuk *Box-Behnken* dipilih untuk memaksimumkan tindak balas (kandungan *eurycomanone*) dari lakaran *response surface* yang terhasil. Kandungan ekstrak *eurycomanone* lebih dipengaruhi oleh suhu (> 100^{o}C) diikuti oleh masa statik. Masa statik yang tinggi (> 11min) akan menyebabkan kandungan *eurycomanone* terdegradasi. Suhu serta masa statik yang lebih rendah akan mengurangkan kecekapan pengekstrakan. Keadaan optimum telah menghasilkan kandungan *eurycomanone* sebanyak 9.21mg/g pada masa statik 8 minit, 5 kitaran statik dan suhu 90 ^{o}C. Kaedah *liquid chromatography coupled with a triple quadrapole and time-of-flight tandem mass spectrometer* (LC-QTOF-MS/MS) digunakan untuk memprofilkan metabolit kecil. Kandungan *quassinoid* utama telah dikenal pasti iaitu *13α (21) -epoxyeurycomanone, eurycomanone, longilactone14, 15β-dihydroxyklaineanone, 6α-hydroxyeurycomalactone, eurycomalide B, laurycolactone A* dan *laurycolactone B.* Kesimpulannya, gabungan kaedah *ASE* dan analisis statistik yang dibentangkan merupakan teknik mudah untuk menyaring pelbagai fotokimia daripada akar Tongkat Ali.