

OPTIMIZATION OF ULTRASOUND AND
ENZYME ASSISTED EXTRACTION OF
EURYCOMANONE FROM *EURYCOMA*
LONGIFOLIA (TONGKAT ALI)

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Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG



SUPERVISOR'S DECLARATION

I hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy (Bio-process Engineering).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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HE YUHAI

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy

Faculty of Chemical Engineering & Natural Resources
UNIVERSITI MALAYSIA PAHANG

June 2018

Dedicated to my dearest wife Asiya Zhou Qin and my son Hamid He Liangzhi

ACKNOWLEDGEMENTS

In the name of The Great Almighty Allah SWT.

My greatest wishes and thanks to The Great Almighty Allah who gave my life and instructs me on the way of life.

My sincere gratefulness and love to my wife, parents, grandparents and siblings for their endless love support and encouragement to me to accomplish my studies.

To the infinite firmness, eagerness and patient guidance of my dearest supervisor Dr. Ahmad Ziad Bin Sulaiman, I would like to express my deepest appreciation and gratitude. Thank you so much for your support, fruitful guidance and time to time feedback.

Deepest affection goes to my fellows especially Dr. Aladdin, Ms Aimi, Ms Adilah. Thanks to them to accompany me in this challenging journey. Great appreciation also goes to Orioner Hightech Sdn. Bhd. for its financial sponsorship during my study.

My unlimited thanks to all lab fellows, seniors during my PhD study for their pleasant company, conversations, help, guidance and friendship.

My best regards to Universiti Malaysia Pahang and their rewarding of Doctoral Study Scheme (DSS) to assist financially my studies. Thank you so much to all of the technical staffs at Faculty of Chemical and Natural Resources Engineering.

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LIST OF SYMBOLS

V_{\max}	Maximum rate of enzyme activity
ϵ	Extinction coefficient
Δ	Change

LIST OF ABBREVIATIONS

ABTS	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
DoE	Design of Experiment
EC	Enzyme commission
EDTA	Ethylene diamine tetra acetic acid
FCCCD	Face Centred Central Composite Design
OFAT	One-Factor-At-a-Time
rpm	Revolution per minute
RSM	Response Surface Methodology
RT	Room temperature
HPLC	High Performance Liquid Chromatography
SEM	Scanning Electron Microscopy
LC-MS	Liquid Chromatography and Mass Spectroscopy
P	Power of sonication
I	Intensity of sonication
A	Area of probe
UV	Ultraviolet

OPTIMIZATION OF ULTRASOUND AND ENZYME ASSISTED EXTRACTION
OF EURYCOMANONE FROM *EURYCOMA LONGIFOLIA* (TONGKAT ALI)

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy

Faculty of Chemical Engineering & Natural Resources
UNIVERSITI MALAYSIA PAHANG

June 2018

ABSTRAK

Pengekstrakan Eurycomanone dari *E. longifolia* secara konvensional lazimnya dilakukan melalui kaedah rebusan, di mana kualiti produk yang dihasilkan serta masa pengekstrakan adalah tidak sempurna dan tidak efektif. Pendedahan kepada pelbagai kondisi proses yang tidak ampuh biasanya akan menghasilkan produk sampingan atau metabolit. Bagi menangani masalah ini, kajian akan dilakukan ke atas kaedah pengekstrakan alternatif yang berkesan di mana ianya mampu meningkatkan hasil dan kualiti produk yang telah diekstrak. Pengekstrakan *E. longifolia* di dalam kajian ini telah dijalankan menggunakan beberapa pendekatan pengekstrakan, termasuklah kaedah bantuan ultrasonik dan enzim. Pelbagai faktor dalam proses pengekstrakan telah dikaji menggunakan OFAT dan dioptimumkan menggunakan FCCCD dalam RSM. Kaedah pengekstrakan konvensional menggunakan rebusan telah dikaji dan didapati ianya menunjukkan prestasi yang lebih baik berbanding kaedah Soxhlet, di mana sebanyak 0.237% hasil Eurycomanone telah dihasilkan. Walaupun pengekstrakan Soxhlet mencapai hasil yang signifikan, proses pengekstrakannya tidak dapat dioptimumkan berikutan dengan produk yang dihasilkan telah rosak disebabkan oleh suhu tinggi yang digunakan di dalam sistem Soxhlet tersebut. Hasilnya, kaedah rebusan telah digunakan bagi menentukan keberkesanan kaedah pengekstrakan menggunakan ultrasonik, laccase dan cellulase. Antara kaedah yang digunakan bagi pengekstrakan Eurycomanone adalah; kaedah bantuan ultrasonik, kaedah bantuan enzim cellulase, kaedah bantuan enzim laccase, kaedah gabungan enzim cellulose-laccase, kaedah gabungan ultrasonik-enzim cellulase, kaedah gabungan ultrasonik-cellulase-laccase dan kaedah gabungan ultrasonik-enzim laccase. Kaedah pengekstrakan bantuan ultrasonik menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.294%; kaedah bantuan enzim cellulase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.20%; kaedah bantuan enzim laccase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.215%; kaedah gabungan enzim cellulose-laccase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.176%; kaedah gabungan ultrasonik-enzim cellulase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.152%; kaedah gabungan ultrasonik-cellulase-laccase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.190%; kaedah gabungan ultrasonik-cellulase-laccase menghasilkan kepekatan Eurycomanone yang optimum sebanyak 0.193%. Kaedah pengekstrakan bantuan ultrasonik menghasilkan kepekatan eurycomanone yang paling tinggi iaitu 0.294% di mana faktor utamanya adalah disebabkan oleh kadar pembentukan peronggaan dan kerosakan yang dihasilkan dari kadar intensiti proses sonifikasi. Proses pengekstrakan menggunakan bantuan enzim tidak menunjukkan hasil yang baik, tetapi tempoh pengekstrakan telah dapat dikurangkan kepada 1 jam iaitu sama dengan masa yang digunakan untuk pengekstrakan bantuan ultrasonik. Oleh itu, dari segi penjimatan masa pengekstrakan, kaedah bantuan enzim laccase boleh dipertimbangkan sebagai salah satu kaedah yang efektif walaupun jumlah produk yang dihasilkan tidak setanding dengan hasil dari kaedah bantuan ultrasonik. Kajian dari SEM menunjukkan kesan yang nyata proses

pengekstrakan ke atas permukaan substrat. Semua proses pengekstrakan mempunyai kesan yang maksimum ke atas bahan pepejal tetapi kesan pengekstrakan bantuan ultrasonik adalah lebih jelas berbanding kaedah-kaedah pengekstrakan yang lain. **Kesimpulannya**, penggunaan kaedah pengekstrakan bantuan ultrasonik untuk mengekstrak keluar eurycomanone dari *E. longifolia* boleh diklasifikasikan sebagai salah satu pendekatan yang efektif dan produktif.

ABSTRACT

Eurycomanone extraction from *E. longifolia* is conventionally done using decoction method, but most times, the product quality, yield, and extraction duration are not ideal. Exposure to unfavorable process conditions usually results in the formation of product metabolites. To address these problems, the alternative extraction methods that increases the product yield ought to be investigated. In this study, several approaches, including ultrasound and enzyme-assisted methods were studied. Different parameters for the extraction process were screened using OFAT and optimized using FCCCD of RSM. The conventional method of decoction and Soxhlet were studied for eurycomanone extraction, and decoction was found of better performance which gives eurycomanone yield of 0.237 %. The Soxhlet extraction achieved a significant model while the process wasn't able to be optimized because of the degradation of the final product due to the high temperature employed in Soxhlet system. Thus, decoction was used as the control for indicating that if the use of ultrasound, laccase and cellulase could make a better yield of eurycomanone. The methods studied for the extraction of eurycomanone are ultrasound-assisted extraction, cellulase enzyme-assisted extraction, laccase enzyme-assisted extraction, cellulase enzyme-laccase enzyme combined extraction, laccase enzyme-ultrasound combined extraction, cellulase enzyme-ultrasound combined extraction, cellulase enzyme-laccase enzyme-ultrasound combined extraction. **Ultrasound-assisted** extraction gives optimum eurycomanone yield of 0.294 %; **Cellulase enzyme-assisted** makes optimum eurycomanone yield of 0.20 %; **Laccase enzyme-assisted** extraction makes optimum eurycomanone yield of 0.215 %; **Cellulase-laccase enzyme** makes optimum eurycomanone yield of 0.176 %; **Cellulase enzyme-ultrasound combined extraction** makes optimum eurycomanone yield of 0.152 %; **Laccase enzyme-ultrasound combined extraction** makes optimum eurycomanone yield 0.190 %; **Cellulase-laccase-ultrasound combined extraction** makes optimum eurycomanone yield of 0.193 %. The ultrasound-assisted extraction method yielded the highest eurycomanone (0.294%) which was mainly due to disruption of the cell wall by the cavitation formation and collapse of the sonication process. The enzyme assisted extraction didn't show a better extraction, however, it is remarkable that the use of laccase made the extraction period shortened to 1h which same as the time used by the ultrasound assisted extraction. Thus, in consideration of time saving in the extraction, laccase enzyme-assisted extraction should also be considered even though its yield is not as high as ultrasound-assisted extraction. SEM studies revealed a considerable effect of the extraction processes on the surface of the substrates. All the extraction processes had considerable effects on the solid material but the effects of ultrasound assisted extraction were more pronounced. **In conclusion**, the use of ultrasound assisted methods for the extraction of eurycomanone from *E. longifolia* could be considered effective and friendly approaches.

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