ULTRASOUND AND ENZYMATIC MEDIATED EXTRACTION OF VITEXIN AND ISOVITEXIN COMPOUNDS FROM *FICUS DELTOIDEA* LEAVES

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We 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 Master of Engineering in Chemical.

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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 citation 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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Dedicated to my parents and siblings for always standing by my side and nurturing me with love and support

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LIST OF SYMBOLS

- μ micro
- A Ampere
- eV Electron volts
- Hz Hertz
- m/z Mass-to-charge ratio
- Pa Pascal
- s seconds
- V Volt
- W Watt

LIST OF ABBREVIATIONS

AE	Aqueous extraction
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BEH	Ethylene Bridged Hybrid
DAD	Diode Array Detector
EC	Enzyme Commission
EnAE	Enzymatic-assisted extraction
FE-SEM	Field Emission-Scanning Electron Microscopy
HPLC	High Performance Liquid Chromatography
i.d.	Inner diameter
MS	Mass Spectrometry
RPC	Reversed-phase column
rpm	Revolutions per minute
sp.	species
UAE	Ultrasound-assisted extraction
UAEnE	Ultrasound-assisted enzymatic extraction
UPLC	Ultra-Performance Liquid Chromatgraphy
UV-Vis	Ultraviolet-visible
var.	variety
WHO	World Health Organization

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ABSTRAK

Ficus deltoidea atau lebih dikenali sebagai 'Mas Cotek' merupakan salah satu tumbuhan herba yang berharga di Malaysia disebabkan oleh kandungan bahan aktifnya yang bermanfaat dan penggunaannya sebagai ramuan ubat-ubatan tradisional. Kajian ini memberi tumpuan dalam mengkaji prestasi sistem ultrasonik menggunakan tiga rejim kitaran operasi iaitu: 10%, 20% dan 40% pada keamatan 8.66 W/cm² dalam pengekstrakan bahan aktif daripada daun F. deltoidea melalui perlaksanaan kedua-dua sistem ultrasonik dan enzim dalam menentukan rejimen sonikasi dan mekanisma tindakbalas yang sesuai untuk meningkatkan produktiviti ekstrak berbanding dengan kaedah tradisi. Kajian ini turut menekankan kaedah mengenalpasti bahan aktif iaitu vitexin dan isovitexin yang terdapat di dalam ekstrak daun F. deltoidea. Daun F. deltoidea betina digunakan dalam kajian ini disebabkan oleh ketersediaan dan kandungan bahan aktif yang tinggi terdapat di dalamnya. Hasil optimum sebatian vitexin telah direkodkan sebanyak 1.067 ± 0.001 (% w/w) dari nisbah sampel-air 1:10 (g/mL), suhu 50 °C, kitaran operasi 10% dan kepekatan enzim 0.4% pada jam ke-3 pengekstrakan dijalankan. Manakala, bagi sebatian isovitexin, hasil optimum yang direkodkan adalah sebanyak 0.374 ± 0.028 (%w/w) dari nisbah sampel-air 1:20 (g/mL), suhu 50 °C serta kitaran opearsi 10% pada jam ke-3 pengekstrakan dijalankan. Pencirian morfologi ke atas permukaan ekstrak daun kering turut dijalankan menggunakan FE-SEM. Sampel daun kering yang telah diekstrak menggunakan bantuan ultrasonik dan juga enzim menunjukkan permukaan yang lebih pecah dan berlubang berbanding sampel yang dirawat secara konvensional. Hasil eksperimen yang diperolehi dalam kondisi terbaik adalah sejajar dengan nilai yang diramalkan, di mana ultrasonik dan enzim adalah merupakan proses yang lebih efektif berbanding pengekstrakan didihan secara konvensional. Penambahbaikan yang berjaya dilakukan ke atas kaedah pengekstrakan menggunakan ultrasonik dan enzim di dalam kajian ini perlu dilanjutkan untuk mengenalpasti lebih banyak sebatian fenolik dari lain-lain bahagian pokok F. deltoidea dan ianya tersedia sebagai kunci informasi kepada skala kajian yang lebih besar pada masa yang akan datang.

ABSTRACT

Ficus deltoidea or 'Mas Cotek' is regarded as one of the most precious herb plants in Malaysia due to its content of beneficial active compounds and appreciation as a traditional remedy. This research focused on the investigation of the performance of ultrasound using three sonication duty cycle regimes: 10%, 20% and 40% at an intensity of 8.66 W/cm² in the extraction procedure of active compounds from F. deltoidea leaves by the implementation of both ultrasound and enzyme to determine the sonication regiments and their mechanism reactions that might be suitable to improve the productivity of extraction comparatives to control. It is also focus on the identification of active compounds that present in the leaves of F. deltoidea, especially of vitexin and isovitexin. Due to the availability and abundancy of active compounds, female leaves of F. deltoidea were chosen to be used in this study. The optimum yield recorded for vitexin compound was 1.067 ± 0.001 (%w/w) from; 1:10 (g/mL) sample-to-water ratio, 50 °C temperature, 10% duty cycle, 0.4% enzyme concentration at 3rd hour of extraction. While for isovitexin compound, the optimum value recorded was 0.374 ± 0.028 (%w/w) from; 1:20 (g/mL) sample-to-water ratio, 50 °C temperature, 10% duty cycle at 3rd hour of extraction. The morphological characterization of the extracted dried leaves particles performed using FE-SEM showed a slight ruptures and more voids on the surface of ultrasound-assisted and enzymatic-mediated extraction sample when compared to conventional extracted sample. The experimental values under best conditions were in good consistent with the predicted values, which suggested that sonication and enzymatic treatment are more efficient process in comparison to conventional boiling extraction. The improvement on the extraction method using ultrasound and enzymatic approaches in the present study should be extended to the identification of more phenolic compounds from various parts of F. deltoidea plants and serves as key information for larger scale study in the future work.

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LIST OF PUBLICATIONS AND CONFERENCES

Published paper and abstract:

- 1. Nur Aimi Syairah Mohd Abdul Alim, A.Z. Sulaiman and Azilah Ajit. (2016). Application of Ultrasound on the Extraction of Vitexin from *Ficus deltoidea* leaves. ARPN Journal of Engineering and Applied Sciences. 11(4), 2199-2204. http://www.arpnjournals.org/jeas/research_papers/rp_2016/jeas_0216_3626.pdf
- 2. Nur Aimi Syairah Mohd Abdul Alim, Azilah Ajit and A.Z. Sulaiman. (2015). Abstract. The Effects of Ultrasound on the Extraction of *Ficus deltoidea* leaves. https://waset.org/abstracts/28134

Attended conferences:

- 5th International Conference on Biotechnology for the Wellness Industry (ICBWI 2014), 10-11 June 2014, Universiti Teknologi Malaysia, Kuala Lumpur, Malaysia. (Poster presentation)
- 2. ICCSE 2015: 18th International Coference on Chemical Science and Engineering, 21-22 April 2015, Istanbul, Turkey.
- 3. International Conference on Fluids and Chemical Engineering (FluidsChE 2015), 25-27 November 2015, Adya Hotel, Langkawi Island, Malaysia.

In-progress submission in Innovative Food Science and Emerging Tehnologies:

Nur Aimi Syairah Mohd Abdul Alim, Sulaiman, A.Z. and Ajit, A. (2016). Effect of unified system of ultrasound and enzyme on the extraction of vitexin and isovitexin compounds from *Ficus deltoidea* var. *deltoidea* leaves extracts.

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