OPTIMIZING MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS, *IN VITRO* ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES OF THE EXTRACTS FROM BITTER LEAVES (*Vernonia amygdalina*) AND PURPLE FLEABANE (*Vernonia cinerea*)

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SUPERVISOR'S DECLARATION

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 Doctor of Philosophy.

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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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ABSTRACT

Diabetes mellitus is a common metabolic disorder, resulting from the inability of a body in responding to the high level of glucose in the bloodstream. The uses of synthetic drugs pose adverse effects on human systems, thus, sourcing alternative from plants is imperative. Therefore, the optimization of microwave-assisted extraction factors in the recoveries of phenolic compounds from bitter leaf (*Vernonia amygdalina*) and purple fleabane (*Vernonia cinerea*) leaves as compared with extracts through Soxhlet extraction method had been investigated in this study. The *in vitro* antioxidant and anti-diabetic activities of the extracts at optimized conditions were as well studied. The effects of MAE factors which include irradiation time, microwave power level, temperature, feed-to-solvent ratio, and ethanol concentration on the extraction yields (% w/w); total phenolic contents, TPC (mg GAE/g d.w.) and total flavonoid contents, TFC (mg QE/g d.w.) were studied using the one-factor-at-a-time (OFAT) experimental method. The two-level factorial design was used to further screen and determine significant extraction factors of MAE. Then, four significant MAE factors namely irradiation time, microwave power, feed-to-solvent ratio, and ethanol concentration were optimized using a face-centered central composite design (FCCCD) of response surface methodology (RSM). Likewise, OFAT was employed to determine the optimal yields through Soxhlet extraction by considering extraction time (1-4 h), feed-to-solvent ratio (1:10-1:25 g/mL) and ethanol concentration (20-80% v/v) as the factors for both plant samples. At the optimized conditions of MAE and Soxhlet extraction, the extracts were characterized using liquid chromatography-mass spectrometry quadrupole time of flight (LC-Q-TOF-MS), Fourier transform infrared spectroscopy (FTIR), and inductively coupled plasma mass spectrometry (ICP-MS). The optimal MAE conditions for *V. amygdalina* leaves were at irradiation time of 4 min, microwave power level of 558 W; feed/solvent of 1:10 g/mL, and ethanol concentration of 76% v/v. Meanwhile, for *V. cinerea* leaf, the optimal MAE conditions were at irradiation time of 2 min, microwave power level of 444 W, feed/solvent of 1:14 g/mL, and ethanol concentration of 47% v/v. Moreover, the optimal recoveries of extraction yields, TPC and TFC from *V. amygdalina* and *V. cinerea* leaves were obtained using Soxhlet extraction technique at 2 h of extraction time, feed-to-solvent of 1:20 g/mL and ethanol concentration of 60% v/v. The obtained results reflected that MAE can recover higher yields of extracts with the significant quantities of phenolic compounds, antioxidant and anti-diabetic activities from *V. amygdalina* and *V. cinerea* leaves as compared with Soxhlet extraction. In addition, LC-Q-TOF-MS analysis confirmed that the identified phenolic compounds through optimized MAE were higher as compared to Soxhlet extraction technique. Moreover, FTIR spectra showing the presence of phenolic compounds were obtained for optimized MAE compared to Soxhlet extraction technique. From the ICP-MS analysis, the presence of mineral elements (potassium, magnesium, calcium, and chromium) in the extracts showed that MAE technique possesses a higher capacity to extract more nutrients compared to Soxhlet extraction. In addition, the extracts from both plant samples showed stronger antioxidant activities whereby the higher quantities were obtained through MAE. The anti-diabetic inhibitory effects were higher than acarbose (a standard drug) for *V. amygdalina* leaf. However, the anti-diabetic inhibitory effects were closer to that of acarbose for *V. cinerea* leaves. Thus, the extracts from MAE of *V. amygdalina* and *V. cinerea* leaves can be inferred as the promising sources of antioxidants and anti-diabetic medicines for pharmaceutical and functional food industries.

ABSTRAK

Diabetes mellitus adalah gangguan metabolik biasa, hasil daripada ketidakupayaan badan yang bertindak balas kepada tahap glukosa yang tinggi dalam aliran darah. Oleh itu, pengoptimuman faktor pengekstrakkan menggunakan mikrowave dalam pemulihan sebatian fenolik daripada daun pahit (*Vernonia amygdalina*) dan fleabane ungu (*Vernonia cinerea*) dibandingkan dengan kaedah pengekstrakan Soxhlet telah disiasat dalam kajian ini. Antioksidan dan aktiviti anti-diabetes daripada ekstrak pada keadaan dioptimumkan dikaji juga. Kesan faktor MAE termasuk masa pengekstrakkan, tahap kuasa gelombang mikro, suhu, sampel-pelarut nisbah dan kepekatan etanol kandungan fenolik, TPC (mg GAE/g dw) dan jumlah kandungan flavonoid, TFC (mg QE/g dw) telah dikaji dengan menggunakan (OFAT), kaedah satu faktor pada satu masa eksperimen. Kedua-dua peringkat reka bentuk faktorial digunakan untuk melanjutkan paparan dan menentukan faktor-faktor signifikasi pengekstrakkan MAE. Begitu juga, OFAT telah digunakan untuk menentukan hasil yang optimum melalui pengekstrakan Soxhlet dengan mempertimbangkan masa pengekstrakan (1-4 h), sampel-to-pelarut nisbah (1:10-1:25 g/mL) dan kepekatan etanol (20-80% v/v) sebagai faktor untuk kedua-dua sampel tumbuhan. Pada keadaan yang optimum MAE dan pengekstrakan Soxhlet, ekstrak dicirikan menggunakan cecair masa spektrometri quadrupole kromatografi-jisim penerbangan (LC-Q-TOF-MS), jelmaan Fourier spektroskopi inframerah (FTIR), dan induktif ditambah spektrometri jisim plasma. Keadaan MAE optimum bagi *V. amygdalina* adalah pada masa pengekstrakkan 4 min, tahap kuasa gelombang mikro 558 W; sampel/pelarut 1:10 g/mL, dan kepekatan etanol 76% v/v. Sementara itu, untuk daun *V. cinerea*, keadaan MAE optimum adalah pada masa pengekstrakkan 2 min, tahap kuasa gelombang mikro 444 W, sampel/pelarut 1:14 g/mL, dan kepekatan etanol 47% v/v. Selain itu, pemulihan optimum hasil pengekstrakan, TPC dan TFC dari *V. amygdalina* dan *V. cinerea* daun telah diperolehi dengan menggunakan teknik pengekstrakan Soxhlet pada 2 h masa pengekstrakan, sampel-to-pelarut 1:20 g/mL dan etanol kepekatan 60% v/v. Keputusan yang diperolehi mencerminkan bahawa MAE boleh mendapatkan hasil ekstrak dari *V. amygdalina* dan *V. cinerea* yang lebih tinggi dengan kuantiti sebatian fenolik, antioksidan dan aktiviti anti-diabetes yang banyak berbanding pengekstrakan Soxhlet. Di samping itu, analisis LC-Q-TOF-MS mengesahkan 93 sebatian fenolik dikenal pasti daripada ekstrak daun *V. amygdalina* melalui MAE berbanding dengan ekstrak diperolehi daripada pengekstrakan Soxhlet (22 sebatian fenolik). Selain itu, sebanyak 80 dan 13 sebatian fenolik telah dikenal pasti dari ekstrak daun *V. cinerea* yang diperolehi melalui MAE yang dioptimumkan dan Soxhlet pengekstrakan, masing-masing. FTIR spektrum menunjukkan kehadiran sebatian fenolik yang tinggi untuk MAE yang dioptimumkan berbanding dengan teknik pengekstrakan Soxhlet. Daripada analisis ICP-MS, kehadiran unsur-unsur mineral (kalium, magnesium, kalsium, dan kromium) dalam ekstrak menunjukkan bahawa teknik MAE mempunyai kapasiti yang lebih tinggi untuk mendapatkan lebih banyak nutrien berbanding pengekstrakan Soxhlet. Ekstrak *V. amygdalina* dan daun *V. cinerea* melalui MAE menunjukkan kesan dos yang paling tinggi kekangan anti-diabetes pada 1.0 mg/mL terhadap α-amilase dan enzim α-glucosidase. Kesan-kesan dos berkenaan adalah lebih tinggi daripada acarbose (dadah standard) untuk daun *V. amygdalina*. Walau bagaimanapun, kesan anti-diabetes tersebut adalah lebih kurang sama dengan acarbose untuk daun *V. cinerea*. Oleh itu, ekstrak *V. amygdalina* dan *V. cinerea* daun daripada MAE boleh disimpulkan sebagai sumber menjanjikan antioksidan dan ubat-ubatan anti-diabetes untuk industri farmaseutikal dan makanan berfungsi.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

A	Irradiation time (min)
Adj. R^2	Adjusted coefficient of determination
B	Microwave power (W)
C	Extraction temperature (°C)
D	Feed-to-solvent ratio (g/mL)
E	Ethanol concentration (% v/v)
m/z	Mass-to-charge ratio
R^2	Coefficient of determination
Y_1	Response for extraction yield
Y_2	Response for total phenolic content
Y_3	Response for total flavonoid content

LIST OF ABBREVIATIONS

ABTS	2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CV	Coefficient of variation
DPPH	2,2-diphenyl-picryl hydrazyl
DNA	Deoxyribonucleic acid
DNS	3,5-dinitro salicyclic acid
FCCCD	Face-centered central composite design
FTIR	Fourier Transform infrared spectrometry
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
LC-Q-TOF-MS	Liquid chromatography-mass spectrometry
MAE	Microwave-assisted extraction
NMR	Nuclear Magnetic Resonance
OFAT	One-factor-at-a-time
pNPG	p-nitro-phenyl-α-D-glucopyranoside
PRESS	Predicted residual of sum squares
RNA	Ribonucleic acid
RSM	Response surface methodology
TFC	Total flavonoid content
TPC	Total phenolic content

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