CHARACTERIZATIONS OF SAPONIN AND PHENOLIC BIOACTIVE COMPOUNDS EXTRACTED FROM FENUGREEK SEED AND ALOE VERA LEAVES VIA MICROWAVE-ASSISTED EXTRACTION METHOD

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

College of Engineering

UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2021

ACKNOWLEDGEMENTS

First and foremost, I would like to thank almighty Allah for giving me health, knowledge, strength, opportunity and ability to undertake my studies and complete this work.

During this journey, the Almighty Allah also blessed me to carry out this work under the supervision of Professor Dr. Abdurahman Hamid Nour, a role model, a friend, an inspiration and a hand of support. He was always eager to support me and provide me invaluable guidance, supports, great ideas, and suggestions that have helped me to complete this thesis. His knowledge and advice supported me to never give-up when faced life's problems during my studies. This thesis would not have been possible without his guidance and supports. I would really like to thank him for his assistance during my PhD journey. My special thanks also go to my co-supervisor Professor Dato Dr. Rosli Bin Mohd Yunus for his supports and guidance during my PhD studies.

I would also like to thank my husband Dr. Fahim Fayaz for his supports and helps in entire of my PhD journey. My thanks also go to my parents for their prayers and giving me the strength while being far from them. Thanks to my brothers and my only sister for encouraging and supporting me.

My special thanks go to University Malaysia Pahang for rewarding me Doctoral Research Scheme (DRS) for financial assistance. I would like to thank the honorable Dean, Deputy Dean, faculty staff and technical staff of Faculty of Chemical and Process Engineering Technology, University Malaysia Pahang.

ABSTRAK

Dalam kajian ini, potensi benih fenugreek dan Aloe vera sebagai sumber saponin, fenolik dan antioksidan telah dikaji. Pengekstrakan dibantu oleh ketuhar gelombang mikro (MAE) dan pengekstrakan Soxhlet digunakan untuk mendapatkan ekstrak. Kesan faktor eksperimen MAE seperti masa pengekstrakan (2-12 min), kuasa gelombang mikro (300-700 W), kepekatan etanol (20-100%), nisbah pelarut (F:S) (1:8-1:16 dan 1:18-1:22 g/mL) dan suhu pengekstrakan (40-80°C) dinilai menggunakan (OFAT). Faktor pengekstrakan Soxhlet termasuk masa pengekstrakan (1-5 h), kepekatan etanol (20-100%) dan F: S (1:14-1:24 g/mL) juga dikaji. Keputusan menunjukkan hasil pengekstrakan yang lebih tinggi, jumlah kandungan saponin (TSC) dan jumlah kandungan fenolik (TPC) diperoleh melalui MAE berbanding pengekstrakan Soxhlet dalam penjimatan masa. Hasil maksimum dalam pengekstrakan Soxhlet diperoleh pada 3 jam masa pengekstrakan, kepekatan etanol 60% dan nisbah 1:20 g/mL F:S yang 19.35 ± 0.75%, 125.04 ± 1.55 mg DE/g d.w. dan 60.13 ± 2.04 mg GAE/g d.w. untuk benih fenugreek dan 22.45 ± 0.76%, 44.78 ± 1.01 mg OAE/g d.w. dan 49.99 ± 0.56 mg GAE/g d.w. untuk Aloe vera. Penyaringan terhadap "factors via two-level factorial design" dijalankan untuk parameter MAE. Keputusan menunjukkan bahawa kepekatan etanol dan suhu pengekstrakan adalah faktor yang paling penting dan paling tidak penting dalam mencapai hasil maksimum. Pemilihan tahap faktor untuk proses pengoptimuman diperoleh berdasarkan faktor pra-penilaian (OFAT). Faktor optimum yang terbaik adalah masa pengekstrakan (2-4 min), kuasa gelombang mikro (500-700 W dan 400-600 W), kepekatan etanol (40-80% dan 20-60%), F:S (1:8-1:12 dan 1:18-1:22 g/mL) dan suhu ialah 70 °C. Kondisi optimum untuk benih fenugreek dan Aloe vera menghasilkan hasil pengekstrakan, TSC dan TPC berada pada 2.84 min, 572.50 W, 63.68%, dan 1:9 g/mL. Berdasarkan keadaan optimum, hasil pengekstrakan, TSC dan TPC benih fenugreek adalah 26.04 ± 0.88%, 195.89 ± 1.07 mg DE/g dw, 81.85 ± 0.61 mg GAE/g dw, dan ekstrak daun Aloe vera di MAE adalah 2.79 min, 478.95 W, 43.38% etanol, dan 1:19 g/mL. Berdasarkan kepada kondisi tersebut, hasil ekstrak daun TSC dan TPC dari Aloe vera adalah 36.17 ± 1.13%, 65.89 ± 0.77 mg OAE/g d.w dan 73. 05 ± 1.05 mg GAE/g d.w. Ekstrak yang diperoleh melalui MAE dan Soxhlet juga diuji untuk kapasiti antioxidant melalui DPPH dan ABTS, struktur dan ikatan menggunakan FTIR dan kajian morfologi menggunakan SEM. Keputusan menunjukkan bahawa ekstrak yang diperoleh melalui MAE menunjukkan kapasiti antioksida yang lebih tinggi dengan nilai IC_{50} yang rendah (195.27 ± 0.56 μg/mL; DPPH), (157.92 ± 1.11 μg/mL; ABTS), 12 puncak yang dikenal pasti dalam FTIR untuk benih fenugreek dan (275 ± 1.45 μg/mL; DPPH) (215.58 ± 0.57 μg/mL; ABTS), 11 puncak di FTIR dan masing-masing tekstur kemas dan tekstur terbuka melalui SEM. Walau bagaimanapun, ekstrak Soxhlet (224.47 ± 0.77 μg/mL; DPPH), (199.67 ± 0.96 μg/mL; ABTS) untuk benih fenugreek dan (305.79 ± 0.66 μg/mL; DPPH), (263.29 ± 1.21 μg/mL, ABTS), dengan 6 puncak yang dikenal pasti melalui FTIR dan liang yang tidak tertutup dan liang tertutup ditunjukkan melalui SEM pada kedua-dua tanaman. kajian kinetik dan sifat dielektrik untuk MAE juga dilakukan. Hasil LC-QTOF-MS dari optimum ekstrak mengesahkan kehadiran 58 saponin dan 27 fenolik dalam benih fenugreek dan 29 saponin dengan 32 fenolik dalam ekstrak Aloe. Optimum ekstrak juga menunjukkan sifat surfaktan seperti pembasahan, pengurangan ketegangan permukaan air, sifat berbuih dan emulsifikasi. Oleh itu, ekstrak ini boleh menjadi sumber saponin, fenolik, antioksidan dan pengemulsi semula jadi untuk makanan, kosmetik dan produk farmaseutikal.

ABSTRACT

In this study, the potential of fenugreek seed and Aloe vera leaves as a source of saponins, phenolics and antioxidants were investigated. Microwave-assisted extraction (MAE) and Soxhlet extraction (SE) were used to obtain the extracts. The effects of experimental factors in MAE such as extraction time (2-12 min), microwave power (300-700 W), ethanol concentration (20-100%), feed-to-solvent ratio (1:8-1:16 and 1:18-1:22 g/mL) and extraction temperature (40-80 °C) were evaluated using one-factor-at-a-time (OFAT), respectively. The SE factors including extraction time (1-5 h), ethanol concentration (20-100%) and feed-to-solvent ratio (1:14-1:24 g/mL) were also investigated. Results indicated the higher extraction yield, total saponin content (TSC) and Total phenolic content (TPC) were obtained via MAE compared to SE in a time saving process. The maximum yields in SE were obtained at 3 h of extraction time, 60 % ethanol concentration and 1:20 g/mL F:S ratio which were 19.35±0.75%, 125.04±1.55 mg DE/g d.w. and 60.13±2.04 mg GAE/g d.w. for fenugreek seed and 22.45±0.76%, 44.78±1.01 mg OAE/g d.w. and 49.99±0.56 mg GAE/g d.w. for Aloe vera leaves, respectively. Further screening of the factors via two-level factorial design was carried out for MAE parameters. Results indicated that ethanol concentration and extraction temperature were the most and least significant factors in achieving maximum recoveries of the yields, respectively. The selection of factor levels for optimization process was obtained based on the pre-evaluation of factors (OFAT). The best points for optimizing the factors were extraction time (2-4 min), microwave power (500-700 W and 400-600 W), ethanol concentration (40-80% and 20-60%), feed-to-solvent ratio (1:8-1:12 and 1:18-1:22 g/mL) and constant temperature of 70 °C, respectively. The optimal MAE conditions for fenugreek seed and Aloe vera leaves extraction yield, TSC and TPC were at 2.84 min, 572.50 W, 63.68%, and 1:9 g/mL. Based on the optimum condition, the responses of extraction yield, TSC and TPC of fenugreek seed were 26.04 ± 0.88%, 195.89 ± 1.07 mg DE/g d.w., 81.85 ± 0.61 mg GAE/g d.w, and for Aloe vera leaves extracts in MAE were 2.79 min, 478.95 W, 43.38% ethanol, and 1:19 g/mL. Where, based on these conditions, the extraction yield, TSC and TPC of Aloe vera leaves extract were 36.17 ± 1.13%, 65.89 ± 0.77 mg OAE/g d.w and 73. 05 ± 1.05 mg GAE/g d.w, respectively. The extracts obtained via MAE and SE were also tested for its antioxidant capacity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,20-azino- bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), structure and bonding using Fourier transform infrared (FTIR) and morphological studies using SEM. Results indicated that extracts obtained via MAE showed higher antioxidant capacity with low IC_{50} values of (195.27 ± 0.56 µg/mL; DPPH), (157.92 ± 1.11 µg/mL; ABTS), 12 identified peaks in FTIR for fenugreek seed and (275 ± 1.45 µg/mL; DPPH) (215.58 ± 0.57 µg/mL; ABTS), 11 peaks in FTIR and more wrapped and opened texture via scanning electron microscope (SEM), respectively. However, in SE it was (224.47 ± 0.77 µg/mL; DPPH), (199.67 ± 0.96 µg/mL; ABTS) for fenugreek seed and (305.79 ± 0.66 µg/mL; DPPH), (263.29 ± 1.21 µg/mL; ABTS), with 6 identified peaks via FTIR and closed pores showed via SEM in both plants, respectively. kinetic studies and dielectric properties for MAE were also carried out. The LC-QTOF-MS result of optimized extracts also confirmed the presence of 58 saponins and 27 phenolic compounds in fenugreek seed and 29 saponin with 32 phenolic compounds in Aloe extract. The optimized extracts also indicated surfactant properties such as wetting, reduction of water surface tension, foaming and emulsification properties. Thus, these extracts can be a promising source of saponins, phenolics, antioxidants and natural co-emulsifier for food, cosmetics and pharmaceutical products.

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R2	R- square
Adj. R2	Adjacent coefficient of determination
C	Carbon
A	Alpha
Θ	Theta
Y	Response
β_0	Constant term
β_i	Coefficient of linear factor
β_{ii}	Coefficient of quadratic parameter
β_{ij}	Coefficient of interaction parameters
ε'	Dielectric constant
ε''	Dielectric loss
τ	Relaxation time
ω	Angular frequency in radians per second
ε_∞	Complex permittivity
ε_s	Static permittivity
λ	Wavelength
λ_0	Wavelength in free space

LIST OF ABBREVIATIONS

MAE	Microwave-assisted extraction
UAE	Ultrasonic-assisted extraction
DCM	dichloromethane
TSC	Total saponin content
TPC	Total phenolic content
DPPH	2,2-diphenyl-1-picrylhydrazyl (DPPH),
ABTS	2,20-azino- bis (3-ethylbenzothiazoline-6-sulfonic acid
RSM	Response surface methodology
ANOVA	Analysis of variance
CV	Coefficient of variation
FCCCD	Face-centred central composite design
FTIR	Fourier transform infrared (FTIR) spectroscopy
SEM	Scanning electron microscopy
LC-QTOF-MS	Liquid chromatography-mass spectrometry quadrupole time-of-flight
OFAT	One-factor-at-a-time
DNA	Deoxyribonucleic acid
WHO	World health organization
DOE	Design expert
D_P	Penetration depth

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