EXTRACTION OF BIOACTIVE COMPOUNDS FROM HIBISCUS AND JASMINE FLOWERS USING MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD) AND HYDRODISTILLATION (HD) METHODS

HESHAM HUSSEIN ALAADDIN RASSEM

DOCTOR OF PHILOSOPHY

UNIVERSITI MALAYSIA PAHANG

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.

____

(Supervisor's Signature)

Full Name : PROF. DR. ABDURAHMAN HAMID NOUR

Position : PROFESSOR

Date :

____

(Co-supervisor's Signature)

Full Name : PROF. DATO' DR. ROSLI BIN MOHD YUNUS

Position : PROFESSOR

Date :

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.

____

(Student's Signature)

Full Name : HESHAM HUSSEIN ALAADDIN RASSEM

ID Number : PKC15008

Date :

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HESHAM HUSSEIN ALAADDIN RASSEM

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ABSTRAK

Penggunaan minyak pati asal semulajadi baru-baru ini mendapat banyak perhatian dalam banyak bidang seperti perasa makanan, racun perosak dan industri farmaseutikal. Walau bagaimanapun, batasan penggunaan kaedah pengekstrakan konvensional yang sedia ada adalah penggunaan masa yang sedia ada, kos yang tinggi, penggunaan tenaga yang tinggi, kehilangan potensi sebatian yang tidak menentu dari minyak pati serta kebimbangan alam sekitar. Oleh itu, teknik pengekstrakan inovasi baru diperlukan untuk mengatasi batasan-batasan ini. Dalam kajian ini, minyak pati telah diekstrak daripada bunga Hibiscus dan bunga Jasmine dengan menggunakan hidrodistilasi konvensional (HD) dan kaedah hidrodistilasi dibantu oleh mikro-konvensional bukan konvensional (MAHD). Pengaruh pra-rawatan pada bunga Hibiscus dan bunga Jasmine sebelum pengekstrakan diselidiki. Juga, perubahan dalam morfologi serbuk bunga Hibiscus dan serbuk bunga Jasmine sebelum dan selepas pengekstrakan oleh kedua-dua kaedah diperhatikan melalui SEM. Gangguan kelemahan minyak pati bunga Hibiscus diperhatikan untuk MAHD berbanding dengan HD. Ini dikaitkan dengan pengagihan haba yang berkesan yang boleh diperolehi daripada MAHD. Kecekapan teknik pengekstrakan MAHD dibandingkan dengan HD dari segi komposisi kimia dan aktiviti biologi minyak diperoleh serta implikasi kos proses pengekstrakan. Untuk mewajarkan prestasi teknik MAHD, tiga faktor utama mempengaruhi seperti nisbah bahan pelarut-untuk-tumbuhan, masa pengekstrakan dan daya gelombang penyinaran dianalisis. Analisis faktor-faktor ini pada mulanya dijalankan menggunakan satu faktor pada satu masa. Selain itu, penyaringan dan pengoptimuman faktor-faktor telah dijalankan dengan bantuan perisian pakar reka bentuk melalui analisis faktorial dan reka bentuk komposit pusat, masing-masing. Keadaan optimum yang diperoleh melalui CCD untuk MAHD adalah masa pengekstrakan 120 min, 8: 1 metanol kepada nisbah bahan mentah dan 300 kuasa gelombang penyinaran. Hasil maksimum yang diperolehi dari bunga Hibiscus dan Jasmine adalah 1.25 % dan 1.21 %, masing-masing. Sebaliknya, parameter pengekstrakan untuk HD adalah 160 minit masa pengekstrakan, 8: 1 metanol kepada nisbah bahan mentah dan kuasa operasi berterusan 350 W. Hasil maksimum yang diperolehi dari bunga Hibiscus dan Jasmine untuk HD adalah 1.15 % dan 1.13 %, masing-masing. Minyak penting yang didapati pada keadaan operasi optimum untuk kedua-dua kaedah adalah tertakluk kepada analisis kualitatif yang lebih lanjut. Analisis komposisi dilakukan melalui spektroskopi massa kromatografi gas. Sejumlah 37 sebatian didapati dalam kedua kaedah pengekstrakan (MAHD dan HD) untuk minyak bunga Hibiscus yang disokong oleh analisis fourier mengubah spektroskopi inframerah. Bagaimanapun, sebatian aktif yang terdapat dalam minyak bunga Hibiscus (asid etanimidic, etil ester) menunjukkan nilai min 31.48 ± 0.2 dan 29.23 ± 0.2 untuk MAHD dan HD, masing-masing. Begitu juga dengan pengumpulan 10 sebatian diperolehi kaedah pengekstrakan untuk minyak bunga Jasmine yang disokong oleh analisis FTIR. Walau bagaimanapun, sebatian aktif yang terdapat dalam minyak bunga Jasmine (2-Phenylthiolane) menunjukkan nilai min 57.31 ± 0.1 dan 57.21 ± 0.1 untuk MAHD dan HD, masing-masing. Selain itu, sifat-sifat antioksidan yang dipamerkan oleh bunga Hibiscus dan bunga Jasmine yang diperolehi melalui MAHD telah dinilai untuk menentang DPPH Radikal Scavenging Assay. Menariknya, ekstrak mentah yang diperoleh melalui MAHD menunjukkan nilai IC_{50} 0.7 ppm dan 5.15 ppm bagi bunga Hibiscus dan bunga Jasmine, masing-masing. Ini menunjukkan bahawa teknik MAHD sesuai untuk mendapatkan bunga Hibiscus dan bunga Jasmine dan minyak yang diperolehi boleh menawarkan manfaat farmaseutikal yang hebat.

ABSTRACT

The use of natural origin essential oil has recently gained much attention in many fields such as food flavoring, pesticides and in pharmaceutical industries. However, limitations to the use of existing conventional extraction methods are the inherent time consumption, high cost, high power consumption, potential loss of volatile compounds from essential oil as well as environmental concerns. Therefore new innovation extraction techniques are required to overcome these limitations. In this research, essential oil was extracted from Hibiscus flower and Jasmine flower by using conventional hydrodistillation (HD) and non-conventional microwave assisted hydrodistillation (MAHD) methods. The influence of pre-treatment on the Hibiscus flower and Jasmine flower prior to extraction was investigated. Also, changes in morphology of the Hibiscus flower powder and Jasmine flower powder before and after extraction by the two methods was observed through SEM. Milder disruption of Hibiscus flower oil gland were observed for MAHD compared to HD. This is associated with the effective heat distribution obtainable from MAHD. The efficiency of MAHD extraction technique was compared with HD in terms of chemical composition and biological activity of the oil obtained as well as cost implication of the extraction process. To justify the performance of MAHD technique, the three main influencing factors such as solvent–to-plant material ratio, extraction time and irradiation microwave power were analyzed. Analysis of these factors was initially carried out using one factor at a time method. Furthermore, screening and optimization of the factors was conducted with the help of Design expert software via factorial analysis and central composite design, respectively. The optimum conditions obtained through CCD for MAHD is 120 min extraction time, 8:1 of methanol to raw material ratio and 300 W irradiation microwave power. The maximum yield obtained from Hibiscus and Jasmine flowers is 1.25 % and 1.21 %, respectively. On the other hand, the extraction parameters for HD are 160 min extraction time, 8:1 of methanol to raw material ratio and a constant operating power 350 W. The maximum yield obtained from Hibiscus and Jasmine flowers for HD is 1.15 % and 1.13 %, respectively. The essential oil obtained at the optimum operating conditions for both methods was subjected to further qualitative analysis. Compositional analysis was conducted through gas chromatography-mass spectrometer. A total of 37 compounds were found in both extraction methods (MAHD and HD) for Hibiscus flower oil which were supported by fourier transforms infrared spectroscopy analysis. However, the active compound present in Hibiscus flower oil (Ethanimidic acid, ethyl ester) manifested a mean value of 31.48 ± 0.2 and 29.23 ± 0.2 for MAHD and HD, respectively. Similarly, the collection of 10 compounds was obtained both extraction methods for Jasmine flower oil which were supported by FTIR analysis. However, the active compound present in Jasmine flower oil (2-Phenylthiolane) manifested a mean value of 57.31 ± 0.1 and 57.21 ± 0.1 for MAHD and HD, respectively. In addition, the antioxidant properties exhibited by Hibiscus and Jasmine flowers crude obtained through MAHD was evaluated against DPPH Scavenging Radical Assay. Interestingly, the crude extract obtained through MAHD shows IC_{50} value of 0.7 ppm and 5.15 ppm for Hibiscus and Jasmine flowers, respectively. This indicates that the MAHD technique is suitable for obtaining volatile oils from Hibiscus flower and Jasmine flower and the oils obtained can offer great pharmaceutical benefits.

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LIST OF SYMBOLS

Φ	Volume fraction
Ƒ	Frequency
ρ	Density
Ƭ	Temperature (^{o} C)
tan δ	Dielectric loss tangent
P	Microwave power distribution per volume unit
Λ	Wavelength
E	Electric field strength
µm	Micrometer
$-\alpha$	Negative axial
$+\alpha$	Positive axial
D_p	Penetration depth
C_P	Heat capacity
q_{MW}	Volume of heat generation
n_i	Number of interactions

LIST OF ABBREVIATIONS

AACC	American association for clinical chemistry
ANOVA	Analysis of variance
BBD	Box-Behken design
CCD	Central composite design
CAMD	Compressed air microwave distillation
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
EM	Electromagnetic
FMASE	Focused microwave assisted soxhlet or solvent extraction
FTIR	Fourier transform infrared spectrometry
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally response as safe
HD	Hydrodistillation
MAE	Microwave-assisted extraction
MAHD	Microwave-assisted hydrodistillation
MASD	Microwave accelerated steam distillation
MDG	Microwave dry-diffusion and gravity process
MHG	Microwave hydrodiffusion and gravity
MSD	Microwave steam distillation
MSDF	Microwave steam diffusion
NIST	National institute of standards and technology
OFAT	One factor at a time
PMAE	Portable microwave assisted extraction
PTFE	Polytetraflouro ethylene
RSM	Response surface methodology
RT	Retention time
SEM	Scanning electron microscopy
SFE	Supercritical fluid extraction
SFME	Solvent free microwave extraction
TDS	Triple distribution system
UAE	Ultrasound-assisted extraction
VMHD	Vacuum microwave hydrodistillation

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