

UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF SOLID LIQUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM ORTHOSIPHON STAMINEUS BENTH LEAVES

MOHD FARHAN B ABDUL RAZAK

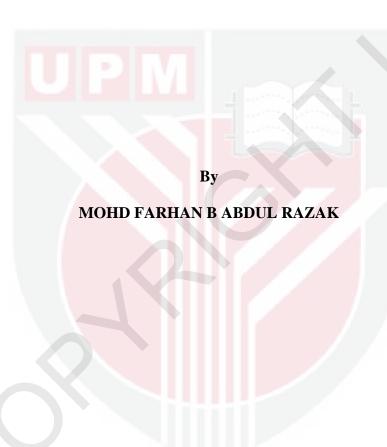
FK 2012 113

OPTIMISATION OF SOLID LIQUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM ORTHOSIPHON STAMINEUS BENTH LEAVES

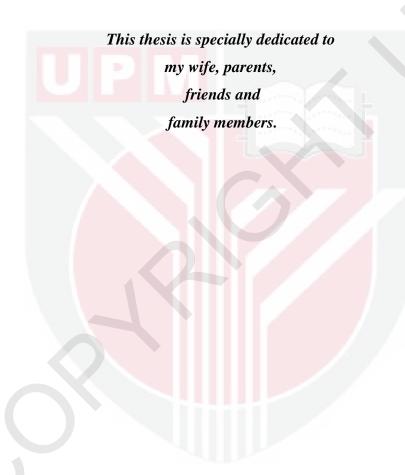


MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF SOLID LIQUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM *ORTHOSIPHON STAMINEUS* BENTH LEAVES



Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of Requirements for the Master Degree



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

OPTIMISATION OF SOLID LIQUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM ORTHOSIPHON STAMINEUS BENTH LEAVES

By

MOHD FARHAN ABDUL RAZAK

June 2012

Chairman: Professor Luqman Chuah Abdullah, PhD

Faculty: Engineering

Orthosiphon stamineus, also known as Misai Kucing, is one of the invaluable medicinal

plants originated from Southeast Asia. O. stamineus leaves were used in plenty of

applications related to the medicinal purposes and are believed to cure certain disease such as

hypertension, gout and fever. However, very little research done with regards to ascertain

optimum extraction process and quality extract of *O. stamineus* leaves.

This research studied a solid-liquid extraction (SLE) process which are involved in

processing of bioactive extract from O. stamineus leaves. Different experiments were carried

out to determine the effects of various operating parameters on the qualitative and

quantitative aspects of O. stamineus leaves. Rosmarinic acid (RA) and Sinensetin (SEN) were

selected as the quality indicators of the product because these two compounds were played a

major role in the bioactivities including anti- inflammatory, anti- oxidant and anti- pyretic of

O. stamineus leaves.

Water is most suitable solvent for extracting O. stamineus leaves compared to ethanol, ethyl

acetate, and hexane. This was because water gave the highest extraction yield as well as

iii

quality extract that related to RA and SEN. Besides, water also indicated highest antioxidant and anti- inflammatory compared to other solvents.

The optimum extraction condition of *O. stamineus* leaves was determined as 60°C and the ratio of water to solid is 10:1 (ml:g) to avoid degradation of SEN based on analysis using Response Surface Methodology (RSM) technique. of 6 hours were found to be optimum based on analysis of Response Surface Methodology (RSM). Two hours of extraction duration was selected as optimum extraction time based on kinetic study. The activation energy of the extraction is 36.57 kJ mol⁻¹.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai

memenuhi keperluan untuk ijazah Master of Science

MENG- OPTIMUMKAN KADAR SEBATIAN AKTIF YANG WUJUD DI DALAM DAUN ORTHOSIPHON STAMINEUS BENTH MENGGUNAKAN TEKNIK

PENGEKSTRAKAN PEPEJAL- CECAIR

Oleh

MOHD FARHAN ABDUL RAZAK

June 2012

Pengerusi:

Professor Luqman Chuah Abdullah, PhD

Fakulti:

Kejuruteraan

Orthosiphon stamineus, juga dikenali sebagai Misai Kucing, adalah salah satu daripada

tumbuhan ubatan yang bernilai dan berasal dari Asia Tenggara. Banyak kajian telah

dijalankan menggunakan daun O. stamineus dan hasil kajian tersebut menunjukkan bahawa

daun O. stamineus dipercayai dapat menyembuhkan penyakit tertentu seperti tekanan darah

tinggi, gout dan demam. Walau bagaimanapun, amat sedikit penyelidikan yang dilakukan

mengenai proses penentuan kadar optimum semasa proses pengekstrakan dan kualiti bagi

daun O. stamineus.

Kajian ini dijalankan menggunakan kaedah pengekstrakan pepejal-cecair (SLE) bertujuan

untuk mengkaji kadar bahan aktif dalam daun O. stamineus. Kajian dijalankan menggunakan

pemboleh ubah yang berbeza bagi menentukan kaitan antara pemboleh ubah tersebut kepada

kualiti dan kualitatif aspek bagi daun O. stamineus. Asid Rosmarinic (RA) dan Sinensetin

(SEN) telah dipilih sebagai petunjuk kualiti produk kerana kedua-dua bahan aktif ini telah

memainkan peranan utama dalam kajian anti-radang, anti-oksida dan dapat menurunkan suhu

badan.

Berdasarkan kajian ini, air adalah pelarut yang paling sesuai untuk mengekstrak daun *O. stamineus* berbanding etil asetat, etanol dan heksana. Ini adalah kerana air memberi hasil ekstrak yang tertinggi serta menunjukkan kadar kepekatan kedua- dua bahan aktif (penunjuk kualiti) yang tinggi iaitu RA dan SEN. Selain itu, air juga menunjukkan antioksidan dan antiradang yang tertinggi berbanding pelarut-pelarut lain.

Hasil ekstrak yang optimum diperolehi daripada suhu pengekstrakan 60 ° C dan nisbah air kepada pepejal adalah 10:1 (ml:g) berdasarkan analisis Kaedah Respons Permukaan (RSM) bagi mengelakkan degradasi bahan aktif SEN. Dua jam dipilih sebagai masa pengekstrakan yang optimum berdasarkan ujian kinetik yang dijalankan. Tenaga pengaktifan juga dikira dan nilainya adalah 36.57 kJ mol⁻¹.

ACKNOWLEDGEMENT

First of all, I would like to express my gratitude to my advisor and chairman of the supervisory committee, Professor Dr. Luqman Chuah Abdullah, for his advice and guidance in making this research successful. I would also like to thank my supervisory committee members, Associate Professor Dr. Thomas Choong Shean Yaw, and Dr Pin Kar Yong for giving useful ideas and comments throughout the project.

I would also like to extend my thanks to my colleagues from Herbal Technology Center and Natural Product Division of Forest Research Institute Malaysia (FRIM) especially En Zamree Md Shah and Dr Rasadah Mat Ali for their technical assistance and kind knowledge-sharing.

Last but not least, I want to extend my deepest gratitude to my parents and family members, my friends especially Mohd Raside and Rohaizuan Rossili for their encouragement and support. Special thanks to my wife, Mdm Nazira Mahmud for standing by me through my study.

I certify that a Thesis Examination Committee has met on 11th Jun 2012 to conduct the final examination of Mohd Farhan Bin Abdul Razak on his thesis entitled "Optimisation of Solid Liquid Extraction of Bioactive Compounds From *Orthosiphon stamineus* Benth Leaves" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science (Biochemical Engineering).

Members of the Thesis Examination Committee were as follows:

Mohd Halim Shah Bin Ismail, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Chairman)

Zurina Binti Zainal Abidin, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Internal Examiner)

Mohd Amran Bin Mohd Salleh, PhD

Faculty of Engineering Universiti Putra Malaysia (Internal Examiner)

Ramlan Bin Abd Aziz, PhD

Professor
Faculty of Chemical Engineering
Universiti Teknologi Malaysia
(External Examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement of the Master Degree. The members of the Supervisor Committee were as follows:

Luqman Chuah Abdullah, PhD

Professor Faculty of Engineering Universiti Putra Malaysia (Chairman)

Thomas Choong Shean Yaw, PhD

Professor Faculty of Engineering Universiti Putra Malaysia (Member)

Pin Kar Yong, PhD

Research Officer
Herbal Technology Centre
Natural Product Division
Forest Research Institute Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the 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 Putra Malaysia or other institutions.

MOHD FARHAN ABDUL RAZAK Date: 11 June 2012

TABLE OF CONTENTS

			Page		
ABS ACF APP DEC LIST LIST	ROVA CLARA F OF TA F OF FI	LEDGEMENT L	iii v vii viii x xiii xiv		
CHA	APTER				
1	INTI	RODUCTION			
_	1.1		1		
	1.2		3		
	1.3	Orthosiphon stamineus Benth	4		
	1.4	Problem statements	6		
	1.5	Objectives	6		
	1.6	Scope of research	7		
2	LITI	LITERATURE REVIEW			
	2.1	O. stamineus leaves	8		
	2.2	Traditional use of O. stamineus leaves	9		
	2.3	Beneficial bioactivity of O. stamineus leaves	9		
		2.3.1 Anti- inflammatory	9		
		2.3.2 Anti- oxidant activity	11		
		2.3.3 Anti- pyretic potential	11		
	2.4	2.3.4 Hepatoprotective agent	12		
	2.4	Rosmarinic acid and sinensetin	12		
	2.5 2.6	Safety of OS Extraction	14 14		
	2.0	2.6.1 SFE	15		
		2.6.2 SLE	15		
	2.7	Phytochemicals content	15		
	2.8	Type of Solvents	16		
	2.9	Extraction temperature	19		
	2.10	Process duration	20		
	2.11	Ratio of solvent to solid	22		
	2.12	Response surface methodology (RSM)	22		
	2.13	Activation energy, E _a	26		
3	MET	THODOLOGY			
	3.1	Raw material	27		
	3.2	Chemicals and Reagents 2			
	3.3	Optimization of SLE of OS leaves			
		3.3.1 Experiments on varying extraction solvent on extraction yield	28		
		3.3.2. Experiments on quality of OS leaves	29		

		3.3.3	<i>In-vitro</i> Anti- inflammatory Assays based on different extraction				
			solvent	30			
		3.3.4	In- vitro Anti- oxidant Assays	31			
	3.4	iments for effect of extraction parameter on extraction yield and qua	ality				
		extrac	t	34			
		3.4.1	Experiment for effect of extraction temperature on extract quality	34			
		3.4.2	Experiment for effect of extraction temperature and ratio of solver				
			solid on extraction yield and quality extract	35			
		3.4.3	Experiments for effect of extraction time on extraction yield of OS				
			extract	36			
	3.5	Summ	nary of flow	37			
4	RESULTS AND DISCUSSION						
	4.1	Qualitative and quantitative effects of solvent on SLE of O. stamineus leaves					
		_	Effects of solvent on extraction yield and phytochemicals content				
			extract	38			
		4.1.2	Determination of <i>in vitro</i> anti- inflammatory activity of the O.				
			stamineus extract from different solvents	41			
		4.1.3	Anti- oxidant activity of solvent extracts	42			
	4.2	of O. stamineus leaves					
			Effects of extraction temperature on quality extract of OS leaves	44			
		4.2.2	Effects of temperature and ratio of solvent to solid on the yield of				
			extraction with Response Surface Methodology (RSM)	46			
		4.2.3	Effects of extraction duration based on kinetic study	51			
		4.2.4	Activation energy, E _a	54			
5	CONCLUSION						
	5.1	1 Conclusion		57			
	5.2	Future	Future studies				
REF	EREN	CES		59			
	LIST OF PUBLICATIONS						
	ENDIC			67 68			