UNIVERSITI TEKNOLOGI MARA

SCREENING FOR ANTIOXIDANT ACTIVITY, TOXICITY AND ANTI-DIABETIC EFFECT OF ERYTHROXYLUM CUNEATUM STANDARDIZED AQUEOUS EXTRACT

ROSLINA BINTI ALI

Thesis submitted in fulfilment of the requirements for the degree of **Master of Science**

Faculty of Pharmacy

March 2016

CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 21 December 2015 to conduct the final examination of Roslina binti Ali on her Master of Science thesis entitled "Screening For Antioxidant Activity, Toxicity and Anti-Diabetic Effect of *Erythroxylum cuneatum* Standardized Aqueous Extract" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

Mohamed Salama Mohamed Ahmed Salama, PhD Professor Faculty of Pharmacy Universiti Teknologi MARA (Chairman)

Zabidah Binti Ismail, PhD Professor Faculty of Pharmacy Universiti Teknologi MARA (Internal Examiner)

Kamisah Yusof, PhD Associate Professor Faculty of Medicine Universiti Kebangsaan Malaysia (External Examiner)

SITI HALIJJAH SHARIFF, PhD

Associate Professor Dean Institute of Graduates Studies Universiti Teknologi MARA Date: 28 March 2016

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulation of Universiti Teknologi MARA. It is an original and is the result of my own work, unless otherwise indicated or acknowledgements as referenced work were made accordingly. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been acknowledged with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, with respect to the conduct of my study and research.

Name of student : Roslina binti Ali

Student I.D. No. : 2008264836

Programme : Master of Science (Pharmacology)

Faculty : Pharmacy

Thesis Title : Screening For Antioxidant Activity, Toxicity and Anti-

Diabetic Effect of Erythroxylum cuneatum Standardized

Aqueous Extract.

Signature of Student:

Date : March 2016

ABSTRACT

Erythroxylum cuneatum (Erythroxylaceae) standardized aqueous extract was investigated for its antioxidant and antihyperglycemic activities for its safety in vivo. Dried extract was provided by Forest Research Institute of Malaysia (FRIM). Antioxidant activity was evaluated via total phenolic content assay, 1, 1-diphenyl-2picrylhydrazyl (DPPH) scavenging activity assay, ferric reducing antioxidant power (FRAP) assay, and via inhibitory effect of extract against lipid peroxidation in liver microsomes induced by tert-butylhydroperoxide (tert-BOOH). Safety of extract was determined using Organization for Economic Co-operation and Development (OECD) guidelines acutely and subacutely in mice. Total phenolic content of extract was 0.38 ± 0.15 mg gallic acid equivalent (GAE)/mg dry weight of extract. DPPH assay showed E. cuneatum extract had an EC₅₀ of $6.17 \pm 0.02 \,\mu g/mL$, while the antioxidant references were trolox, gallic acid and quercetin which elicited EC₅₀ values of 3.89 ± 0.09, 0.3 \pm 0.07 and 2.69 \pm 0.07 $\mu g/mL$, respectively. EC₅₀ values for E. cuneatum extract, trolox and gallic acid in the FRAP assay were 3.72 \pm 0.28, 12.09 \pm 0.44 and $18.59 \pm 0.21 \, \mu \text{mol Fe}^{2+}/\text{mg}$ dry weight, respectively. IC₅₀ value for *E. cuneatum* extract, trolox and gallic acid for inhibition of tert-BOOH-induced lipid peroxidation were 31.62 ± 0.10 , 10.00 ± 0.21 and 12.59 ± 0.12 mg/mL, respectively. Antioxidant activity of extract was correlated to total phenolic content. Based on Organization for Economic Co-operation and Development (OECD) guidelines, oral E. cuneatum extract is non-toxic as no adverse effect were seen at levels of more than 5 g/kg following acute oral administration and 1 g/kg following subacute administration in male and female mice. However, when administered acutely via intraperitoneal route, a dose of 300 mg/kg extract was moderately toxic, thus was harmful. In the streptozotocin induced hyperglycemic rat model, E. cuneatum extract (100 and 300 mg/kg, p.o.) administered twice daily elicited a significant decrease in serum glucose level after 3 weeks of treatment. In summary, E. cuneatum standardized aqueous extract given orally was safe in acute and subacute treatment. It has glucose-lowering effect in hyperglycaemic condition and showed high antioxidant activities in four different antioxidant assays.

TABLE OF CONTENTS

		Pages			
CONFIRMATION BY PANEL OF EXAMINERS		ii			
AUTHOR'S DECLARATION		iii			
ABSTRACT ACKNOWLEDGEMENT TABLE OF CONTENTS LIST OF TABLES LIST OF FIGURES LIST OF ABBREVIATIONS LIST OF NOMENCLATURES LIST OF SYMBOLS		iv v vi ix xi xiv xvi xviii			
			CH.	APTER ONE: INTRODUCTION	
			1.1	Background of Study	1
			1.2	Statement of Problem	2
			1.3	Objectives of Study	2
			1.4	Hypotheses	3
			CH.	APTER TWO: LITERATURE REVIEW	
2.1	Antioxidant Studies	4			
2.2	Toxicity Studies	12			
2.3	Anti-diabetic Study	13			
2.4	Phenolic Compounds	16			
2.5	Erythroxylum cuneatum	17			
CH	APTER THREE: MATERIALS AND METHODS				
3.1	Materials				
	3.1.1 Plant Extract	19			
	3.1.1 Experimental Animals	19			