

**UNIVERSITI TEKNOLOGI MARA**

**CYTOLOGICAL AND MOLECULAR  
ANALYSIS OF ANTI-TUMOUR  
BIOACTIVITIES OF *Urginea maritima*  
(L.) BAKER AQUEOUS EXTRACT ON  
HUMAN MALIGNANT NEUROBLASTOMA  
WITH ITS NEUROPROTECTION ABILITY**

**MAATI MUSBAH SALEH ELGHUOL**

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

**Faculty of Applied Science**

December 2016

## ABSTRACT

This study was conducted with the hypothesis that the phytochemical closely related to Libyan medicinal herb, *Urginea maritima* (L.) Baker constituents within water-based extract would own appropriately powerful properties that contribute to intrinsic regulation specific antitumour activities in human malignant SH-SY5Y neuroblastoma cells. The purpose of the present study was to explore the characterisation and identification of the major phytochemical of the *U. maritima* aqueous extract. Additionally, the study also aimed to evaluate their effectiveness on the cellular and molecular mechanism associated with the main anti-tumour criteria. Through utilising several *in-vitro* techniques on both the experimental cell line models involving ( $2 \times 10^4$  to  $1 \times 10^6$  cells/ml) with the quality of  $\geq 90\%$  viability of SH-SY5Y neuroblastoma and RA differentiated (neuron- model) cells. Evaluation of the impact of the active extract ingredients was conducted based on morphological observation, biochemical, cellular function and gene expression profile, and the analysis was carried out for its estimation within different concentrations and durations. Neuroblastoma is a well-known extra-cranial solid tumour and one of the most deadly malignancies in childhood. Indeed, neuroblastoma with high-risk stages is extremely heterogeneous and very aggressive metastases. Although the most intensive multimodal therapies are available, but the key for the successful medical intervention of malignant neuroblastoma is still a challenging task. In this regard, the present investigation data for the first time clearly emphasise the significantly specific anti-tumour activities including viability and proliferation inhibition at a time-dose dependent manner with an estimated  $IC_{50}$  value at  $10\mu\text{g/ml}$ ,  $1\mu\text{g/ml}$  and  $100\text{ng/ml}$  after an incubation at 24, 48 and 72hrs respectively, with less neurotoxicity among the neuron model cells. Efficient apoptosis-causing and the induction of a more pronounced G1 phase arrest. More importantly, the investigation highly supported the ability of novel biological activities of this natural product, as it elucidated that the extract *in-vitro* could directly induce a neuronal differentiation mechanism. Based on the gene expression profiling was performed using the Human Affymetrix microarray module evaluating the biological significance of the *U. maritima* experiments. Following this Gene Ontology (GO) analysis and the major significant pathway through a Database (D.A.V.I.D) was decided. Further, the most promising results were also verified using RT-PCR. The expression profile results established extensive detail on the gene expression that encoded groups of proteins attributed to death receptors interrelated to intrinsic apoptosis pathway involving *bad*, *bid*, *bbc3*, and also elevated caspase-9 for treating malignant SH-SY5Y neuroblastoma population, which are in accordance with our previous findings and confirmed the research hypothesis. Furthermore, the studied extract strengthens cellular machinery correlated with neurogenesis, differentiation and development, bio action due to stimulation of *wnt* signal pathways with overexpression of numerous *wnt* ligands including *wnt3A*, *wnt7A*, *wnt7B* and *wnt11*. Collectively, these novel findings reveal that the active constituents of this unusual natural product, medicinal herb *U. maritima* exhibited dual effects on the neuron cells. Indeed, this preferential ability through diverse bioactivities provides an interesting basis for widespread medical application and a promising therapeutic candidate against neurological diseases, more specifically against neuroblastoma disorders.

## ACKNOWLEDGEMENT

In the Name of Allah, Most Gracious, Most Merciful, all praise and thanks are due to Allah, and peace and blessings be upon His Messenger. I would like to express the most sincere appreciation to those who made this work possible. Advisory members, family and friends.

I would like to thank Associate Professor, Dr. Mohamed Saifulaman Mohamed Said for providing me the opportunity to complete my PhD studies under his valuable guidance, for the many useful advice and discussions, In addition, special thanks are extended to the supervisory committee member; Puan , Mazatulikhma Mat Zain. I am grateful for her, constant encouragement, helpful advice and many fruitful discussions. I wish to thank Dr. Khalilah Abdul Khalil, for her willingness to serve on my supervisory committee.

Thanks and acknowledgements are meaningless if not extended to my father's spirit and pure, mum and sister who deserve my deepest appreciation. I am grateful for the countless sacrifices they made to ensure that I could pursue my dreams and for always being there for me. Real and deepest thanks to them (May ALLAH bless and protect them and may they be blessed with long and healthy life). All praise and thanks words said to them will not be enough.

I am deeply obligated and thankful to my husband Mr. Saleh Ramadan Saleh and my children, for the moral and intellectual support, their understanding, patience and support throughout the period of my study.

Last but not the least, I wish to thank my University (University of Benghazi, Benghazi, Libya) and my country Libya for their contributions in my study either directly or indirectly.

# TABLE OF CONTENTS

<b>CONFIRMATION BY PANEL OF EXAMINERS</b>	ii
<b>AUTHOR' DECLARATION</b>	iii
<b>ABSTRACT</b>	iv
<b>ACKNOWLEDGEMENT</b>	v
<b>TABLE OF CONTENTS</b>	vi
<b>LIST OF TABLES</b>	xii
<b>LIST OF FIGURES</b>	xiv
<b>LIST OF SYMBOLS</b>	xxi
<b>LIST OF ABBREVIATIONS</b>	xxiii
<b>CHAPTER ONE: INTRODUCTION</b>	1
1.1 Research background	1
1.2 Research Objectives	11
1.3 Problem Statement	11
1.4 Research Hypothesis	12
<b>CHAPTER TWO: LITERATURE REVIEW</b>	14
2.1 Introduction	14
2.2 History of Botanical Natural Production	15
2.3 Bioavailability of Phytochemical compound	16
2.4 <i>Urginea maritima</i> (L.) Baker Taxonomy and Distribution	18
2.4.1 Description of Liliaceae	18
2.4.2 Description of <i>Urginea maritima</i> (L.) Baker	19
2.4.3 History of <i>Urginea maritima</i> (L.) Baker	19
2.5 Cardiotonic Steroids	20
2.5.1 Introduction	20
2.5.2 Anti-cancer activities of cardiotonic steroids	21
2.6 Polyphenol compounds	23
2.7 Anti-tumour specific bioactivities	26

2.7.1	Antioxidant	26
2.7.1.1	Introduction	26
2.7.1.2	Antioxidant classification	27
2.7.1.3	The balance between ROS and antioxidants	28
2.7.1.4	Phytochemical antioxidants: Oxidative stress and disease	28
2.7.2	Apoptosis	30
2.7.2.1	Introduction	30
2.7.2.2	Extrinsic pathway	34
2.7.2.3	Intrinsic pathway	36
2.8	Neuroblastoma	41
2.8.1	Neuroblastoma treatment strategy	42
2.8.2	Neuroblastoma derived cell lines	43
2.9	<i>wnt</i> signal Pathway	44
2.9.1	Introduction	44
2.9.2	Role of <i>wnt</i> signalling in the central nerve system	45
2.9.2.1	<i>wnt</i> signalling pathways	45
2.9.2.2	<i>wnt</i> signalling in neuronal development and maturation	51
2.9.2.3	<i>wnt</i> signalling in neuronal differentiation	60
2.9.2.4	<i>wnt</i> members control dendritic morphogenesis	64
2.9.2.5	<i>wnt</i> signalling at central synapses	66
2.9.3	<i>wnt</i> therapeutic target in neurodegenerative diseases	69
<b>CHAPTER THREE: GENERAL RESEARCH METHODOLOGY</b>		70
3.1	Introduction	70
3.2	Materials and methods	70
3.2.1	Preparation of aqueous <i>U. maritima</i> (L. Baker) extract	70
3.2.2	Cells modules and experimental design	71
3.2.2.1	Human malignant neuroblastoma SH-SY5Y cell culture	73
3.2.2.2	RA differentiated (neuron-model) cell culture	73
<b>CHAPTER FOUR: CHARACTERISATION AND IDENTIFICATION OF PHYTOCHEMICAL OF LIBYAN MEDCINAL HERB <i>U. maritima</i> EXTRACT WITH ANTI-TUMOUR ACTIVITIES</b>		75
4.1	Introduction	75