BIOCHEMICAL AND MOLECULAR STUDIES OF SOME PLANTS AS ANTICANCER

By

HUDA MOHAMEDAHMED TALLIMA
B.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agric., Cairo Univ., 2007

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Agricultural Biochemistry)

Department of Agricultural Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

2017

APPROVAL SHEET

BIOCHEMICAL AND MOLECULAR STUDIES OF SOME PLANTS AS ANTICANCER

M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)

By

HUDA MOHAMED AHMED TALLIMA

B.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agric., Cairo Univ., 2007

APPROVAL COMMITTEE

Dr. DOSOUKY A.M.ABD-EL-HALIEM .....
Professor of Biotechnology, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technology Applications, Burj Al-Arab, Alexandria, Egypt.

Dr. AHMED MAHMOUD ABOUL-ENEIN .....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. HANY ABDEL-AZIZ EL-SHEMY .....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. FATEN MOHAMED ABOU-ELELLA
.....
Professor of Biochemistry, Fac. Agric., Cairo University

Date: 28 / 12 / 2017

SUPERVISION SHEET

BIOCHEMICAL AND MOLECULAR STUDIES OF SOME PLANTS AS ANTICANCER

M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)

By

HUDA MOHAMED AHMED TALLIMA

B.Sc. Agric. Sci. (Agric. Biochemistry), Fac. Agric., Cairo Univ., 2007

SUPERVISION COMMITTEE

Dr. HANY ABDEL-AZIZ EL-SHEMY
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. FATEN MOHAMED ABOU-ELELLA
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MAHGOUB MOHAMED AHMED
Professor of Biochemistry, National Organization for Drug Control and Research (NODCAR)

Name of candidate: Huda Mohamed Ahmed Tallima **Degree:** M.Sc.

Title of Thesis: Biochemical and Molecular Studies of some Plants as Anticancer.

Supervisors : Dr. Hany Abdel-Aziz El-Shemy
Dr. Faten Mohamed Abou-Elella
Dr. Mahgoub Mohamed Ahmed

Department : Agricultural Biochemistry

Approval: 28 /12 /2017

ABSTRACT

Medicinal plants play an important role in our life. Therefore, this work aimed to study the antioxidant, anti-inflammatory and anticancer effects of the ethanolic extract of *Rosmarinus officinalis*, *Acacia nolitca*, *Borage officinalis* and *Ammi majus.* Primary compounds were determined in dried plants. Preliminary phytochemical screening of the ethanolic extracts of the four plants demonstrated the presence or absence of terpens, tannins and flavonoids. Quantitative analysis was conducted to determine total phenols and alkaloids for all extracts. Antioxidant activity was determined by DPPH at various concentrations.The effects to quench the DPPH radical was estimated in dose dependent manner for the four plants and the best results were at 1000 ppm for *Rosmarinus officinalis.* The anti-inflammatory activity was determined by using carrageenan induced rats paw edema compared to indomethacin against the four plants with doses 5, 10, 20 mg/kg. The highest effect for both *Rosmarinus officinalis* and *Acacia nolitca* was at 20mg/kg where *Borage officinalis* was at 10 mg /kg and *Ammi majus* was at 5mg/kg. The anticancer activity was determined in A549 lung cancer cell line with the ethanolic extract of *Rosmarinus officinalis*, *Acacia nolitca,* against doxorubcine as a positive control by using neutral red assay. Different concentrations (12.5, 25, 50, 75, 100 ppm) were used. *Rosmarinus officinalis* recorded higher anticancer activity (100%) at 100 ppm with no cell viability while *Acacia nolitca* recorded 28.57%. The expression of apoptosis genes BCl2-Bax was determined. The data showed that BAX expression was increased after treatment by both plants extracts while BCL2 was decreased after treatment by *Acacia nolitca* and *Rosmarinus officinalis* extracts with 0.028 and 0.18 fold change respectively.

TNF-Alpha and 8-hydroxy-desoxyguanosine (8-OHdG) were determined to investigate the anticancer mechanism of the ethanolic extract for both *Rosmarinus officinalis* and *Acacia nolitca* using IC_{50}. *Rosmarinus officinalis* recorded the higher effect.

Key words: *Rosmarinus officinalis, Acacia nilotica*, A549, TNF-alpha, 8-OHdG**,** apoptosis.

DEDICATION

I dedicate this work to my beloved queen mother whose prayers successfully made me the person I am becoming, my father who taught me how to fight with honor till I rich my goal, my sisters Jihad & Eman who supported me a lot to continue and finally my brother Khalid who encouraged me when I faint; your support made me able to gain such success and honor.

Thanks from the earth to the edge of the universe …

ACKNOWLEDGEMENT

First and above of all, I would like to express my great thanks to Allah, for offering me the strength to fulfill this thesis.

I wish to thank my major ***Dr. Hany ElShemy,*** *Professor of Biochemistry, Faculty of Agriculture, Cairo University, for his kind help and appreciation to his indispensable advice and encouragement from the beginning of this work to its end. Also, I wish to express my sincere thanks, deepest gratitude and appreciation to* ***Dr.Faten Abou-ella*** *Professor of Biochemistry, Faculty of Agriculture, Cairo University, and* ***Dr. Mahgoub Mohamed Ahmed*** *Professor of Biochemistry, National Organization for Drug Control and Research for their great help and supervision.*

Dr.Nadia Eltablway *Professor of Biochemistry, National Organization for Drug Control and Research and my dear friend* ***Mariam Motawee*** *who help me to do and understand the statistical analysis.*

Special thanks for ***Fatema al Zahraa S. Abdelrahaman, Abdel-hay Gaber Abu-Hussein*** *for their great effort.*

Thanks a million for ***Samar Salama, Eman,Abo Elrakha, Shaimaa Abdelsadik*** *who help me to write this work.*

I owe my loving thanks to all my faithful friends who are like sparke that lighten my darkness moments….

Endless thanks to my beloving family, my mother, my father, my brother and sisters for their love, patience and support.

Abbreviations

8-OHG: 8 –hydroxy-desoxyguanosine

ABTS: 2,2-azino-bis3-ethylbenzothiazoline-6-sulphonic acid

A. catechu: Acacia catechu

A. majus: Ammi majus.

A. nilotica: Acacia nilotica

APAF: apoptotic protease activating factor.

A.victoriae: Acacia victoriae

ALL : acute lymphoblastic leukemia .

ALP : alkaline phosphatase.

ALT: alanine transaminase.

AP-1 : activator protein 1 .

Apc : adenomatous polyposis coli .

AST: aspartate transaminase.

ATL: the adult T-cell leukemia/lymphoma

BCl2: B-cell lymphoma 2

B. officinalis : Borage officinalis .

BHT: butylated hydroxyl toluene.

CD: cluster of differentiation

CDKs: Cyclin-dependent kinases

cGMP : cyclic guanosine monophosphate

CLP : cecal ligation and puncture .

CNS : central nervous system.

COX-2 : cyclooxygenase -2.

CRI: cancer related inflammation.

DAG : diacylglycerols.

DAL : Dalton's ascitic lymphoma .

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

EGFR : Epidermal growth factor receptor

ELISA : Enzyme linkage immuno-sorbant assay

ERK : extracellular signal –regulated kinase.

FFA : free fatty acids.

FRAP: The Ferric Reducing Power of Plasma.

GC-MS : Gas chromatography mass spectrometry.

GGT : gamma glutamyltransferase.

GSH : Reduced glutathione.

GSHPX: Glutathione peroxidase

HCV : Hepatitis C Virus.

HIV : human immunodeficiency virus .

HIV-1 PR : human immunodeficiency virus -1 protease .

IC_{50} : the half maximal inhibitory concentration .

ICAM: Intercellular Adhesion Molecule

IL: Interleukin
iNOS : inducible nitric oxide synthase

IAPS: inhibitors of apoptosis proteins

JNK : c-jun N-terminal kinase.

LDL : low density lipoprotein

LPS : lipopolysaccharide

LPO:Lipid Peroxidation

MAG : Monoa-Cylglycerols.

MCF7 : Michigan cancer foundation-7.

MMP-9mRNA : matrix metallopeptidase 9.

NADPH: Nicotinamide Adenine Dinucleotide Phosphate-Oxidase

NF-Kappa B : nuclear factor kappa –light chain enhancer of activatied B cells.

NGF : nerve growth factor.

NSCLC : non-small cell lung cancer.

OCD : obsessive compulsive disorder.

PA: phosphatidic acid.

PARP: Poly adeno ribose polymerase

PBMC : peripheral blood mononuclear cell.

PC: phosphatidylcholine.

PGE2: Prostaglandin E2

PMNL : polymorphonuclear leukocytes.

PS : phosphatidylserine.

R. officinalis: Rosmarinus officinalis .

ROS : reactive oxygen species

RT-PCR : Reserve transcriptase polymerase chain reaction.

SCLC : small cell lung cancer.

SMFM : Sucrose methyl 3-formyl-4-methylpentanoate.

SOD: Super Oxide Dismutase

TNF-alpha: tumor necrosis factor alpha.

TAG : triacyl-glycerols .

TKI: tyrosine kinase inhibitor

CONTENTS

CONTENTS (Continued)

LIST OF TABELS

LIST OF FIGURES