

BIOCHEMICAL STUDIES OF SOME PLANTS AS ANTIOXIDANT AND ANTICANCER

By

DALIA AHMED HASHIM MOHAMED

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004

M.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2012

THESIS

**Submitted in Partial Fulfillment of the
Requirements for the Degree of**

DOCTOR OF PHILOSOPHY

In

**Agricultural Sciences
(Agricultural Biochemistry)**

**Department of Biochemistry
Faculty of Agriculture
Cairo University
EGYPT**

2018

APPROVAL SHEET

**BIOCHEMICAL STUDIES OF SOME PLANTS
AS ANTIOXIDANT AND ANTICANCER**

**Ph.D. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

By

DALIA AHMED HASHIM MOHAMED

**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2012**

APPROVAL COMMITTEE

Dr.SAYED SOLIMAN ALSAADANY.....
Professor of Biochemistry, Fac. Agric., Zagazig University

Dr.EMAM ABDEL MOPDY.....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. AMR AHMED MOSTAFA.....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. FOUAD ABDEL REHIM AHMED.....
Professor of Biochemistry, Fac. Agric., Cairo University

Date: 16 / 01 / 2018

SUPERVISION SHEET

**BIOCHEMICAL STUDIES OF SOME PLANTS
AS ANTIOXIDANT AND ANTICANCER**

**Ph.D. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

By

DALIA AHMED HASHIM MOHAMED

**B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
M.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2012**

SUPERVISION COMMITTEE

Dr. FOUAD ABDEL REHIM AHMED
Professor of Biochemistry, Fac. Agric., Cairo University.

Dr. AMR AHMED MOSTAFA
Professor of Biochemistry, Fac. Agric., Cairo University.

Dr. NERMIEN ZAKARIA AHMED
**Researcher Assistant Professor of Biochemistry- Molecular Drug
Evaluation Dept.-National Organization for Drug Control & Research.**

Name of Candidate: Dalia Ahmed Hashim Mohamed **Degree:** Ph.D.
Title of Thesis: Biochemical Studies of Some Plants as Antioxidant and Anticancer.

Supervisors: Dr. Fouad Abdel Rehim Ahmed
Dr. Amr Ahmed Mostafa
Dr. Nermien Zakaria Ahmed

Department: Agricultural Biochemistry

Approval: 16/01/2018

ABSTRACT

Part one: This present study was designed to investigate the antioxidant activity of three ethanolic extracts of *Annona squamosa* (leaves), *Coffea arabica* (beans) and *Moringa oleifera* (leaves) *In-vitro*. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Hydroxyl radical scavenging (OH•), Ferric reducing antioxidant power (FRAP), Total antioxidant capacity (TAC), Catalase (CAT), and Peroxidase enzymes (POX). Total phenolic (TP), Total flavonoids (TF) and Total soluble protein (TSP) contents were determined.

Also, the effects on lysosomal enzymatic activities of the three extracts and *Rutin hydrate* (standard) by two doses of each extract as compared to control group on the four marker lysosomal enzymatic activities namely: acid phosphatase "*ACP*"; β -galactosidase " *β -GAL*"; β -N-acetyl glucosaminidase " *β -NAG*", and β -glucuronidase " *β -GLU*" after 60 and 120 minutes of incubation in rat liver were investigated. The chemical profile and the contents of each extract by HPLC method were also determined.

Part two: The potential anti-proliferation properties of the three ethanolic extracts on human breast carcinoma cell line type (MCF-7) was investigated using neutral red uptake assay for the estimation of the cell viability.

The results showed that, the most potent antioxidant activity was observed for *Coffea arabica* (beans) then *Moringa oleifera* (leaves) followed by *Annona squamosa* (leaves) and the most potent inhibitory effect on lysosomal enzymatic activities was observed for *Moringa oleifera* (leaves) then *Coffea arabica* (beans) followed by *Annona squamosa* (leaves) as compared to *Rutin hydrate* which were dose and enzyme type dependent.

Also, the results showed the *Annona squamosa* (leaves) ethanolic extract was the most inhibitory effect (cytotoxic) on human breast carcinoma cell line type (MCF-7) it caused cytotoxic effect on MCF-7 by IC_{50} of 25.9 μ g/ml followed by *Moringa oleifera* (leaves) (58.6 μ g/ml), but *Coffea arabica* (beans) showed an inhibitory effects by 322.5 μ g/ml and by increasing concentration showed greater effects after 24 hours of incubation. It can be concluded that, the ethanolic extracts of *Annona squamosa* (leaves), *Coffea arabica* (beans) and *Moringa oleifera* (leaves) have potent antioxidant; anti-inflammatory activities, also, they have the most potent anti-proliferation activity on human breast carcinoma cell line type (MCF-7).

Key words: *Annona squamosa*, *Coffea arabica*, *Moringa oleifera*, DPPH, FRAP, TAC, OH, total phenolic, total flavonoids, lysosomal enzymes, HPLC, anticancer (TNF- α), (8-OHdG), Comet assay, Caspase-3.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my soul Mother and Father, as well as to my husband; Mohammed, my sisters; Samah and Zeinab, my brothers; Mohammed, Khaled and Badr for encouragement and I can not find enough words to express my feeling towards them.

Also, I wish to express my deepest gratitude for the constant support, understanding, valuable assistance to carry out this work and love that I received from my friends; Shaimaa Rashidy, Rehab Salah, Shimaa Hassan, Al-Shimaa Ahmed and Hanan Ghanam for their help.

ACKNOWLEDGEMENT

First of all, prayerful thanks to our Merciful "ALLAH" who gives me everything I have.

*Deep thanks are due to **Dr. Fouad Abd Elrehim Ahmed** Professor of Biochemistry, Faculty of Agriculture, Cairo University for suggesting the problem, supervision, continued assistance and his guidance through the course of study and revision of the manuscript of this thesis.*

*I wish to express his deepest gratitude and appreciation to **Dr. Amr Ahmed Mostafa** Professor of Biochemistry, Faculty of Agriculture, Cairo University for valuable advice and helpful discussion throughout the research, criticism, encouragement and continuous assistance. Without his help, this work would not be possible.*

*I wish to express my indebtedness and profound gratitude to **Dr. Nermien Zakaria Ahmed** Researcher Assistant Professor of National Organization for Drug Control & Research "NODCAR"; Department of Molecular Drug Evaluation, Giza, Egypt for her help in this work and suggestions kind encouragement. In addition, thank for her supervision, precious advice and comments.*

LIST OF ABBREVIATIONS

ROS	Reactive Oxygen Species
$O_2^{\cdot-}$	Peroxide Anion
$OH^{\cdot}$	Hydroxyl Radical
$ROO^{\cdot}$	Peroxyl
$RO^{\cdot}$	Alkoxy
$NO^{\cdot}$	Nitric Oxide Radical
$3O_2$	Triplet State Molecular Oxygen
H_2O_2	Hydrogen Peroxide
OH^-	Hydroxyl Radical
RNS	Reactive Nitrogen Species
GPx	Glutathione Peroxidase
CAT	Catalase
vitamin C	Ascorbate
GR	Glutathione reductase
APX	Ascorbate peroxidase
DHAR	Dehydroascorbate reductase
MDR	Multidrug resistance
MPs	Medicinal plants
DPPH	Diphenylpicrylhydrazyl
RIP	Ribosome-inactivating protein
HPLC	High Performance Liquid Chromatography
ACP	Acid Phosphatase
β -GAL	β -Galactosidase

β –NAG	β -N-acetyl glucosaminidas
WHO	World Health Organization
r.p.m	Rotations per minute
FRAP	Ferric Reducing Antioxidant Power
8-OHdG	8-hydroxy-2 deoxyguanosine
TNF- α	Tumor necrosis factor- α
β -GLU	β -Glucuronidase
GR	Glutathione Reductase
EDTA	Ethylenediaminetetraacetic Acid
TCA	Trichloroacetic acid
L.C.	Low Concentration
H.C	High concentration

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	5
1. Oxidative stress	5
2. Antioxidants	10
a. Enzymatic antioxidant	11
b. Non-enzymatic antioxidant	12
3. Anti-inflammatory	14
4. Anti-Cancer	15
5. Medicinal plants as anticancer	20
MATERIALS AND METHODS	30
RESULTS AND DISCUSSION	64
1. Phytochemical screening of <i>Annona squamosa</i> (leaves) <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves)	64
2. The total phenolic , total flavonoid and total soluble protein contents of <i>Annona squamosa</i> (leaves) <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves)	66
3. HPLC Analysis of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves	69
4. Determination of Antioxidant activity	72
c. Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) on DPPH radical scavenging activity	72
a. Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves ethanolic extracts on total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP)	75
b. Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves ethanolic extracts on hydroxyl radical scavenging activity (OH•)	78
5. Antioxidant enzymes activities	80
Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) ethanolic extracts on the activities of catalase and peroxidase activity	80

6. Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) and Rutin on the lysosomal enzymatic activities	83
7. Cell culture	89
a. Anti-proliferative Activity and Cytotoxicity analysis of plant extracts on MCF-7 cells	89
b. Levels of tumor necrosis factor- α (TNF- α) ELISA kit.....	94
c. Levels of 8-hydroxy-2 deoxyguanosine (8-OHdG) ELISA kit	96
d. The DNA damage by Comet assay (single cell gel electrophoresis)	99
e. Values of Caspase-3 determined by colorimetric kit	103
SUMMARY	107
REFERENCES	115
ARABIC SUMMARY	

LIST OF TABLES

No.	Title	Page
1.	Preliminary phytochemical analysis of <i>Annona squamosa</i> (leaves) <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves)	64
2.	The total phenolic, total flavonoid and total soluble protein contents of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves).....	67
3.	Phenolic compound contents in <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves).....	70
4.	Flavonoid compound contents in <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves).....	71
5.	Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves on DPPH radical scavenging activity as compared to Rutin hydrate (standard antioxidant)	73
6.	Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves ethanolic extracts on total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP).....	76
7.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) on Hydroxyl radical scavenging activity	78
8.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) ethanolic extracts on the activities of catalase and peroxidase activities	80
9.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) ethanolic extracts	

and Rutin as standard by two doses (5.0 and 10.0 mg/ml) in comparable against control in-vitro on the four marker lysosomal enzymatic activities "ACP, β -GAL, β -NAG and β -GLU in rat liver lysosomes after 60 minutes of incubation.....	85
10. Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) ethanolic extracts and Rutin as standard by two doses (5.0 and 10.0 mg/ml) in comparable against control in-vitro on the four marker lysosomal enzymatic activities "ACP, β -GAL, β -NAG and β -GLU in rat liver lysosomes after 120 minutes of incubation.....	86
11. Percent cell viability of <i>Moringa oleifera</i> and <i>Annona squamosa</i> of MCF7 cell line	90
12. Percent cell viability of <i>Coffea arabica</i> of MCF7 cell line	90
13. IC ₅₀ values of plants extracts on the cytotoxicity of MCF7 cell line	90
14. Levels of tumor necrosis factor- α (TNF- α) ELISA kit ...	94
15. Levels of 8-hydroxy-2 deoxyguanosine (8-OHdG) ELISA kit.....	96
16. DNA damage by Comet assay (single cell gel electrophoresis).....	99
17. Values of Caspase-3 determined by colorimetric kit ...	103

LIST OF FIGURES

No.	Title	Page
1.	Production of ROS by the cell components (mitochondria, plasma membrane and peroxisomes) and by external sources (industrial factors and pathogens). The DNA oxidative damage can be occurred when the antioxidants are not enough	9
2.	Samples of natural antioxidant plants (a,b,c).....	30
3.	Ethanollic extraction preparation	32
4.	Isolation of lysosomal fraction from rat liver	45
5.	The total phenolic(mg GAE/g sample) (A) , total flavonoid (mg quercetin/g sample) (B) and total soluble protein contents (mg/g FW) (C) of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves).....	68
6.	Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves on DPPH radical scavenging activity as compared to Rutin hydrate (standard antioxidant).....	73
7.	Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves ethanolic extracts on Total Antioxidant Capacity (TAC).....	76
8.	Effects of <i>Annona squamosa</i> leaves, <i>Coffea arabica</i> beans and <i>Moringa oleifera</i> leaves ethanolic extracts on Ferric Reducing Antioxidant Power (FRAP).....	77
9.	Effects of <i>Moringa oleifera</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Annona squamosa</i> (leaves) on Hydroxyl radical scavenging activity	79

10.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) on the activity of catalase	81
11.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) on the activity of Peroxidase	81
12.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) against Rutin (standard) by two doses (5.0 and 10.0 mg/ml) on the four marker lysosomal enzymatic activities "ACP, β -GAL, β -NAG and β -GLU in rat liver after 60 minutes of incubation as compared to control group.....	87
13.	Effects of <i>Annona squamosa</i> (leaves), <i>Coffea arabica</i> (beans) and <i>Moringa oleifera</i> (leaves) against Rutin (standard) by two doses (5.0 and 10.0 mg/ml) on the four marker lysosomal enzymatic activities "ACP, β -GAL, β -NAG and β -GLU in rat liver after 120 minutes of incubation as compared to control group.....	87
14.	Effect of <i>Moringa oleifera</i> leaves extract on cancer cell viability :MCF-7. Cancer cell was incubated with various concentrations of extract (25,50,75,100 μ g/ml).	91
15.	Effect of <i>Coffea arabica</i> beans extract on cancer cell viability :MCF-7. Cancer cell was incubated with various concentrations of extract (100,200,300,400,500,600 μ g/ml)	91
16.	Effect of <i>Annona squamosa</i> leaves extract on cancer cell viability :MCF-7. Cancer cell was incubated with various concentrations of extract (25,50,75,100 μ g/ml).	92
17.	Levels of tumor necrosis factor- α (TNF- α) ELISA kit.....	95
18.	Levels of 8-hydroxy-2 deoxyguanosine (8-OHdG) ELISA kit	97

19.	Effect of negative control leaves on breast cancer cell line	100
20.	Effect of <i>Annona squamosa</i> leaves on breast cancer cell line	100
21.	Effect of <i>Coffea arabica</i> beans on breast cancer cell line	100
22.	Effect of <i>Moringa oleifera</i> leaves on breast cancer cell line.....	100
23.	Determination of Caspase-3 colorimetric kit	103