

ACKNOWLEDGEMENT

I would like to express my gratitude and appreciation to the members of the advisory committee;

Prof. Dr. Abdel Nasser B. Singab, Professor of Pharmacognosy, Vice Dean for the Society Service Affairs and Environmental Development, Faculty of Pharmacy, Ain Shams University for suggesting the research point. Words are not enough to express profound thanks to him for his kind supervision, indispensable advice, valuable comments, constant support, helpful guidance and continuous encouragement throughout the course of study, setting an example to what a dedicated professor, scientist and advisor should be. I am truly proud to be one of his students. His constructive criticism and suggestions helped me to improve this work through the experimental investigations as well as writing and revising the thesis.

As. Prof. Dr. Nahla A. Ayoub, Associate Professor of Pharmacognosy and Head of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, for her kind supervision, indispensable advice, valuable comments. She is a warm and loving person and a true model for all women in science.

Prof. Dr. Eman Noaman Aly, Professor of Biochemistry, National Centre for Radiation, Research and Technology – Atomic Energy Authority who is like a mother for me. No words can express my sincere gratitude and thanks to her for her advice, helpful suggestions, kind supervision, valuable assistance, constant guidance and encouragement throughout the biological study. Her friendly attitude, meticulous observations and efforts have made the work very interesting.

Special thanks go to **Dr. Nermeen S. Youssef**, Lecturer of Pathology, Faculty of medicine, Ain Shams University, for the interpretation of the histopathological slides.

*Many thanks go to my colleagues for their friendly help, interest and valuable comments. Very special thanks to **Dr. Mohamed L. Ashour** for running NMR analysis of the isolated compounds in University of Heidelberg, Germany.*

I cannot find enough words to express my deep love and thanks for my parents: for their continuous care, love and support. They sacrificed their time and efforts to help me to achieve this work.

*Finally, I would like to thank my dear husband **Dr. Albert Victor** for his continuous encouragement, support and most of all his patience for which I am truly grateful.*

*I dedicate this work to my beloved children **Fady and Marlie**, the most precious treasure in my life, whom I give my love, care and I hope that they will always be proud of me.*

Fadia Salah youssef

2010

List of contents

LIST OF CONTENTS

	PAGE
LIST OF FIGURES	iv
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	xi
INTRODUCTION	1
REVIEW OF LITERATURE	3
- Family Brassicaceae (Cruciferae)	3
- Botanical description of family Brassicaceae (Cruciferae)	3
- Botanical description of genus <i>Brassica</i>	4
- Botanical description of <i>Brassica oleracea capitata</i>	5
- Taxonomical classification of <i>Brassica oleracea capitata rubra</i> (red cabbage)	6
- Folk medicinal uses	8
- Economic uses	9
- Biological activities of plants belonging to genus <i>Brassica</i> .	9
- Chemical constituents of genus <i>Brassica</i>	18
MATERIALS, METHODS AND APPARATUS	66
I- Materials and methods for biological studies	66
II- Materials, methods and apparatus for phytochemical screening	89
III- Materials, methods and apparatus for investigation of lipoidal matter of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	98
CHAPTER I	
A) Biological investigation of aqueous acetone, 50% ethanol and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	101
1- <i>In vitro</i> determination of the antioxidant activity using 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging capacity assay	101
2- <i>In vivo</i> determination of acute toxicity of the different extracts	107

List of contents

3- Determination of animals' weights	107
4- <i>In vivo</i> determination of the antioxidant and hepatoprotective activities	108

B) <i>In vitro</i> antioxidant activity of the pure compounds isolated from the ethyl acetate fraction (1-O-sinapoyl glucoside and 3,5,6,7,8-pentahydroxy-4'-methoxyflavone).....	138
--	------------

CHAPTER II

Phytochemical investigation of the ethyl acetate extract of *Brassica*

<i>oleracea capitata rubra</i> (red cabbage) leaves.....	145
---	------------

I- Phytochemical screening of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	145
--	-----

II- Phytochemical fractionation of the ethyl acetate extract of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves... ..	146
--	-----

- Identification of compound **1**: (1-O-sinapoyl glucoside) 150
- Identification of compound **2**: (3,5,6,7,8-pentahydroxy-4'-methoxyflavone) 156

III- Quantitative determination of the pure isolated compounds (1-O-sinapoyl glucoside and 3,5,6,7,8-pentahydroxy-4'-methoxyflavone) in the ethyl acetate extract	159
---	-----

IV- TLC- testing of isothiocyanates in the ethyl acetate fraction	164
---	-----

CHAPTER III

Investigation of the lipoidal matter of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	167
---	------------

1- Preparation of the saponifiable and unsaponifiable matter of the ether extract of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	167
2- GLC analysis of fatty acid methyl esters	167
3- GLC analysis of unsaponifiable matter	170

List of contents

GENERAL SUMMARY	173
CONCLUSIONS AND RECOMMENDATIONS	179
REFERENCES	180
ARABIC SUMMARY	

LIST OF TABLES

Table No.		Page
1	DPPH radical-scavenging activity of fractions from broccoli flower....	16
2	Nitric oxide scavenging effect of fractions from broccoli flower	16
3	DPPH scavenging activities of phenolic fractions extracted from kale <i>Brassica oleracea</i> L. var. <i>acephala</i> DC.) leaf and seed	17
4	Hydroxycinnamic acid esters isolated from genus <i>Brassica</i>	18
5	Chalcone isolated from genus <i>Brassica</i>	23
6	Flavone aglycone isolated from genus <i>Brassica</i>	23
7	Flavonol glycosides isolated from genus <i>Brassica</i>	26
8	Anthocyanins isolated from genus <i>Brassica</i>	44
9	Triterpenoids isolated from genus <i>Brassica</i>	57
10	Glucosinolates isolated from genus <i>Brassica</i>	60
11	<i>In vitro</i> antioxidant activity of aqueous acetone extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	102
12	<i>In vitro</i> antioxidant activity of 50% ethanol extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	103
13	<i>In vitro</i> antioxidant activity of ethyl acetate extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	104
14	<i>In vitro</i> antioxidant activity of methanol extract, different concentrations,	

	of <i>Brassica oleracea capitata alba</i> (white cabbage) leaves	105
15	<i>In vitro</i> antioxidant activity of pure ascorbic acid, different concentrations, used as a reference material.	106
16	Glutathione content in blood of the control group and the different treated groups (n = 6).....	109
17	Superoxide dismutase activity in blood of the control group and the different treated groups (n = 6).....	111
18	Catalase activity in blood of the control group and the different treated groups (n = 6).....	113
19	Lipid peroxidation levels in blood of the control group and the different treated groups (n = 6).....	115
20	Alanine transaminase levels in blood of the control group and the different treated groups (n = 6).....	117
21	Aspartate transaminase levels in blood of the control group and the different treated groups (n = 6).....	119
22	Alkaline phosphatase levels in blood of the control group and the different treated groups (n = 6).....	121
23	Total protein levels in blood of the control group and the different treated groups (n = 6).....	123
24	Total bilirubin levels in blood of the control group and the different treated groups (n = 6).....	125
25	Urea levels in blood of the control group and the different treated groups (n = 6)	127
26	Creatinine levels in blood of the control group and the different treated	

	groups (n = 6).....	129
27	<i>In vitro</i> antioxidant activity of different concentrations of 1- <i>O</i> -sinapoyl glucoside	139
28	<i>In vitro</i> antioxidant activity of different concentrations of 3,5,6,7,8-pentahydroxy-4'-methoxyflavone	140
29	Results of phytochemical screening of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves Brassicaceae.	145
30	Results of the column chromatographic analysis of F-1.....	147
31	Results of the column chromatographic analysis of fraction F-1A	148
32	Results of the column chromatographic analysis of fraction F-1Aa ₂	149
33	Chromatographic and spectral data for compound <u>1</u>	151
34	Chromatographic and spectral data for compound <u>2</u>	157
35	Several concentrations of 1- <i>O</i> -sinapoyl glucoside and their respective peak areas.....	160
36	Several concentrations of 3,5,6,7,8-pentahydroxy-4'-methoxyflavone and their respective peak areas	162
37	Results of gas chromatographic analysis of fatty acid methyl esters of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	168
38	Hydrocarbons and sterols identified in the unsaponifiable matter of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	170

LIST OF FIGURES

Figure No.		Page
1	Photograph of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	7
2	Photograph of flower and fruit of family Brassicaceae.....	7
3	Scheme for preparation of the different extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	67
4	<i>In vitro</i> antioxidant activity of aqueous acetone extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	102
5	<i>In vitro</i> antioxidant activity of 50% ethanol extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	103
6	<i>In vitro</i> antioxidant activity of ethyl acetate extract, different concentrations, of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves.....	104
7	<i>In vitro</i> antioxidant activity of methanol extract, different concentrations, of <i>Brassica oleracea capitata alba</i> (white cabbage) leaves.....	105
8	<i>In vitro</i> antioxidant activity of pure ascorbic acid, different concentrations, used as a reference material.....	106
9	The change in the animals' weight throughout the eight weeks of the experiment	107
10	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood glutathione content (GSH) in different groups of animals.....	109

11	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood superoxide dismutase activity (SOD) in different groups of animals	111
12	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood catalase activity in different groups of animals.....	113
13	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood malondialdehyde level (MDA) in different groups of animals.....	115
14	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on plasma alanine transaminase (ALT) in different groups of animals.	117
15	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on plasma aspartate transaminase (AST) in different groups of animals	119
16	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on plasma alkaline phosphatase (ALP) in different groups of animals.	121
17	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on plasma total protein in different groups of animals.....	123

18	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on plasma total bilirubin in different groups of animals	125
19	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood urea level in different groups of animals	127
20	Effect of aqueous acetone, 50% ethanol, and ethyl acetate extracts of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves, <i>ip</i> administered, on blood creatinine level in different groups of animals.....	129
21, 22	Microphotograph of normal liver section from control group with H&E staining (x400)	131
23, 24, 25, 26	Microphotograph of liver section from CCl ₄ treated group with H&E staining (x400)	132 133
27, 28	Microphotograph of liver section from silymarin and CCl ₄ treated group with H&E staining (x400).....	134
29, 30	Microphotograph of liver section from aqueous acetone extract and CCl ₄ treated group with H&E staining (x400).....	135
31, 32	Microphotograph of liver section from 50% ethanol extract and CCl ₄ treated group with H&E staining (x400)	136
33, 34	Microphotograph of liver section from ethyl acetate extract and CCl ₄ treated group with H&E staining (x400)	137
35	<i>In vitro</i> antioxidant activity of different concentrations of 1-O-sinapoyl glucoside.....	139

List of figures

36	<i>In vitro</i> antioxidant activity of different concentrations of 3,5,6,7,8-pentahydroxy-4'-methoxyflavone	140
37	¹ H-NMR of compound (1): 1-O-sinapoyl glucoside	152
38	¹³ C-NMR of compound (1): 1-O-sinapoyl glucoside	153
39	HSQC of compound (1): 1-O-sinapoyl glucoside	154
40	COSY of compound (1): 1-O-sinapoyl glucoside	155
41	¹ H-NMR of compound (2): 3,5,6,7,8-pentahydroxy-4'-methoxyflavone	158
42	HPLC chromatogram of the ethyl acetate extract dissolved in methanol of HPLC grade.....	159
43	HPLC chromatogram of the ethyl acetate extract enriched with the two isolated compounds dissolved in methanol of HPLC grade.	159
44	HPLC chromatogram of 1-O-sinapoyl glucoside (a pure compound isolated from the ethyl acetate extract) dissolved in methanol of HPLC grade.	160
45	Calibration curve of 1-O-sinapoyl glucoside	161
46	HPLC chromatogram of 3,5,6,7,8-pentahydroxy-4'-methoxyflavone (a pure compound isolated from the ethyl acetate extract) dissolved in methanol of HPLC grade	162
47	Calibration curve of 3,5,6,7,8-pentahydroxy-4'-methoxyflavone..	163
48	TLC plate before spraying with ammoniacal silver nitrate.....	164
49	TLC plate after spraying with ammoniacal silver nitrate	165

List of figures

50	GLC spectrum of the fatty acid methyl esters of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	169
51	GLC spectrum of the unsaponifiable matter of <i>Brassica oleracea capitata rubra</i> (red cabbage) leaves	172

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine transaminase
APCI-MS	Atmospheric Pressure Chemical Ionization–Mass Spectrometry
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BC	Before Christ
b.wt	Body weight
°C	Degree Celsius
CC	Column Chromatography
CF₃COOD-CD₃OD	Deuterated trifluoroacetic acid- deuterated methanol
CDCl₃	Deuterated chloroform
Conc.	Concentration
COSY	Correlated Spectroscopy
¹³C-NMR	Carbon-13-Nuclear Magnetic Resonance
CYP450	Cytochrome P450
<i>d</i>	Doublet
<i>dd</i>	Doublet of doublet
2D	2 dimensional
dil	Dilute
DMSO-<i>d</i>₆	Dimethylsulfoxide-<i>d</i>₆
D₂O	Deuterated water
DPPH	1,1-diphenyl-2-picrylhydrazine
EI-MS	Electron Impact Mass Spectrometry
ESI-MS	Electron Spray Ionization Mass Spectrometry
EC₅₀	Effective concentration by 50%

List of abbreviations

EDTA	Ethylenediaminetetraacetic acid
ev	Electron volt
°F	Degree Fahrenheit
FAB-MS	Fast Atomic Bombardment Mass Spectrometry
FAMe	Fatty Acid Methyl esters
g	Gram
g/dl	Gram/deciliter
g/kg	Gram/kilogram
GLC	Gas Liquid Chromatography
glc.	Glucose
GSH	Glutathione
H&E	Haematoxylin and Eosin
¹HNMR	Proton Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
HPLC-MS	High Performance Liquid Chromatography- Mass Spectrometry.
HREI-MS	High Resolution Electron Impact Mass Spectrometry.
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IC₅₀	Inhibitory concentration by 50%
ID	Internal diameter
<i>ip</i>	Intraperitoneal.
IR	Infra-red
<i>J</i> value	Coupling constant.
Kg	Kilogram
L	Liter
LP	Lipid peroxidation