

CHEMICAL AND PHARMACOLOGICAL STUDIES ON
THE SEEDS OF PRUNUS MAHALEB

A THESIS
SUBMITTED BY

NAHLA SAYED ABDEL-AZIM
BSC CHEMISTRY, CAIRO UNIVERSITY

FOR

MASTER OF SCIENCE

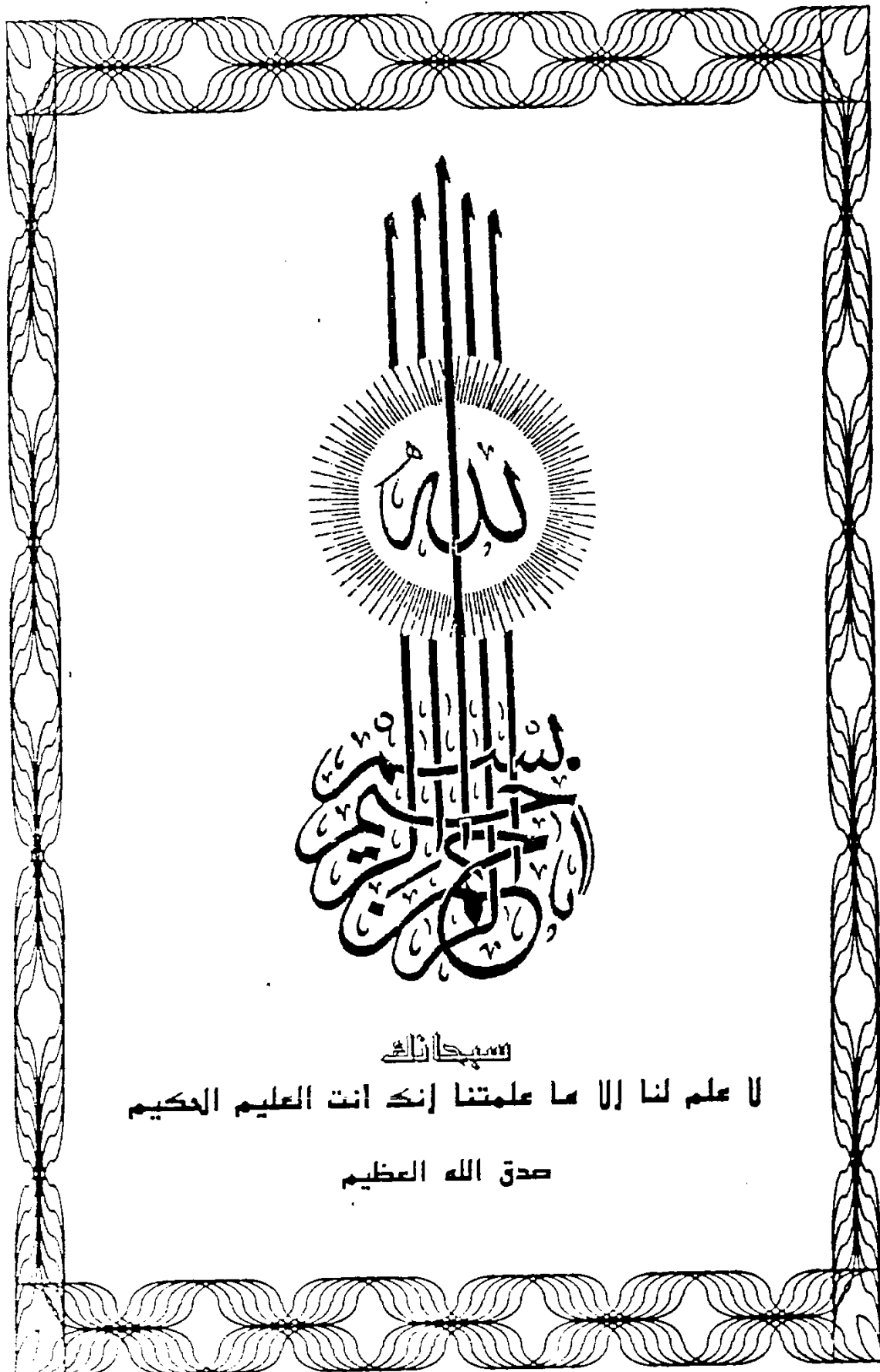
IN

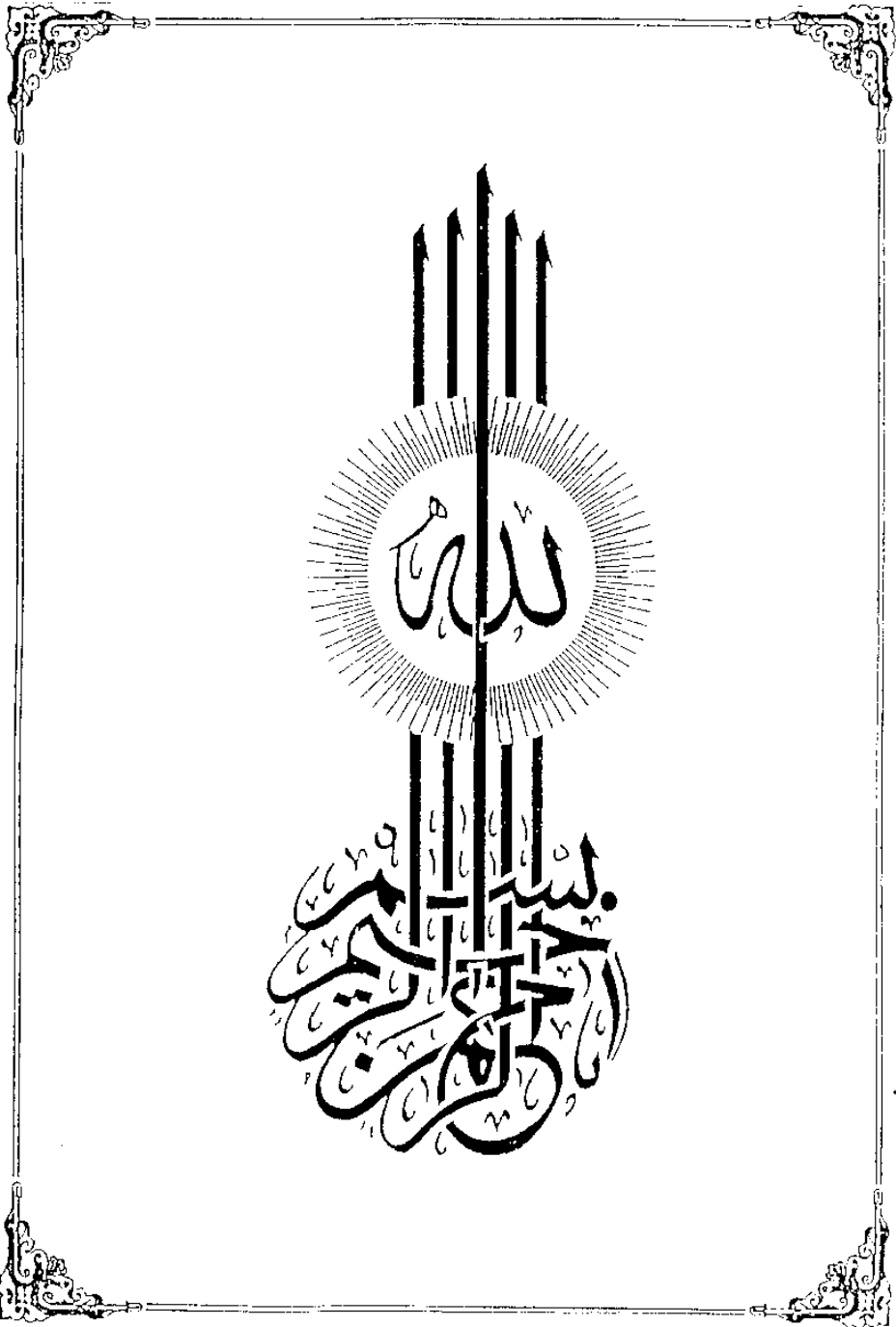
CHEMISTRY

TO

FACULTY OF SCIENCE
AIN SHAMS UNIVERSITY

1991





TO
MY DEAREST FATHER
AND MOTHER

ACKNOWLEDGMENTS

The author would like to acknowledge many people who gave the chance for this work to be done.

I would like to express my deep appreciation to prof. Dr. Abdel-Maged Sammour, Prof. of organic chemistry, Faculty of Science, Ain-Shams University, Cairo, Egypt, for his supervision and guidance.

I am really very grateful to Prof. Dr. Faiza Hammouda, of the Pharmaceutical Science Laboratory, National Research Centre, Cairo, Egypt for her encouragement and constant supervision. I would like also to thank Dr. Nahed Hassen, Ass. Prof. Pharmaceutical Science Laboratory, National Research Centre, Cairo, Egypt.

I owe much gratitude to Prof. Dr. Hidar Ghaleb, Prof. of Pharmacology, Faculty of Medicine, Cairo University for his guidance throughout this work and to Prof. Dr. Kadry Madkour, Prof. of Pharmacology, Faculty of Medicine, Cairo University for his great cooperation and his continuous patience to analyse all the data obtained.

I would further like to acknowledge and thank Prof. Dr. Shams Ismail of the Pharmaceutical Science Laboratory, National Research Centre, Cairo, Egypt, very much for his useful, kind and constant advice and supervision.

I would like especially to thank Dr. C.J.Smith, Biochemistry Research Group, School of Biological Science, University College of Swansea, University of Wales, for giving me the chance to work in his laboratory and allowing me to use all the facilities and equipment required for UV, MS, NMR, and HPLC.

I would like also to thank Dr. Richard Schmidt, School of Pharmacy, Cardiff, University of Wales, for allowing me to work under his project licence to carry out the *in-vivo* Studies.

I am deeply indebted and very grateful to Kato Aromatic S.A.E. and to the Chromatography Laboratory team for their assistance to carry out the GC/MS analysis there.

I would like also to thank Fulton WorldWide, Research Laboratories, New York, U.S.A, for MS and IR analysis.

I would finally like to express my deepest gratitude to all my colleagues at the Pharmaceutical Science Laboratory, National Research Centre, Cairo, Egypt; especially to Dr. Alaa Kamel ; and to the Biochemistry Research Group, School of Biological Science, Swansea, Wales, for their encouragement, useful cooperation and helpful advice all the time throughout the entire work.

CONTENTS

	PAGE
SUMMARY	1
INTRODUCTION.....	4
REVIEW OF LITERATURE.....	6
* The oil	6
* Coumarins	8
* Other Constituents	9
FATTY ACIDS	14
* Introduction	14
* Nomenclature	15
* Classification	18
* Isolation and Preparation	22
* Techniques of Separation	23
COUMARINS	31
* Introduction	31
* Classification	32
* Ultra-violet absorption	46
* Mass spectrometry	47
* Nuclear magnetic resonance	52
* Detection and identification	53
* Biological properties	57
AIM OF WORK	60
PLANT MATERIAL	61
EXPERIMENTAL	62
(1) Phytochemical screening	62
(2) investigation of the oil fraction	68

(2.1) Preparation of the oil	65
(2.2) Chemical treatment of the oil	65
(2.2.1) Saponification process	65
(2.2.2) Extraction of the unsap. matter	66
(2.2.3) GLC of the unsap. matter	66
(2.2.4) Preparation of the total fatty acids	70
(2.2.5) Preparation of the methylesters of the total fatty acids	70
(2.2.6) GLC of the methylesters of the total fatty acids	71
(2.2.7) GC/MS of the methyl esters of the total fatty acids	75
(3) INVESTIGATION OF THE COUMARINS	86
(3.1) Extraction method	86
(3.2) Solvent fractionation process	87
(3.3) Thin-layer chromatography of fraction(B)	90
(3.4) Preparative thick-layer chromatography for fraction (B)	93
(3.5) Identification of the isolated coumarins	94
(3.5.1) Identification of fraction (A)	94
a- UV absorption	94
b- IR absorption	94
c- HPLC	98
d- GC/MS	102
(3.5.2) Identification of fraction (B)	105
a- UV absorption	105
b- IR absorption	105
c- Mass spectrometry	108

d- nuclear magnetic resonance	112
e- Acid hydrolysis of the glucoside	115
(4) INVESTIGATION OF THE MUCILAGE FRACTION	120
(4.1) Preparation of the mucilage	120
(4.2) Hydrolysis of the mucilage	121
(4.3) Qualitative paper chromatography	121
BIOLOGICAL STUDIES	123
AIM OF THE BIOLOGICAL STUDIES	123
MATERIALS AND METHODS	125
a- Acute i.p. lethal toxicity tests	125
b- In-vivo studies	136
RESULTS	139
a- Results of i.p. lethal toxicity tests	139
b- Results of <i>in-vivo</i> studies	150
DISCUSSION	169
REFERENCES	175
ARABIC SUMMARY	

LIST OF TABLES

	PAGE
Table (1) Some of the better known olefinic fatty acids ...	21
Table (2) Preliminary phytochemical screening	64
Table (3) Retention times and relative percentage of the hydrocarbons fraction components	68
Table (4) Retention times and relative percentage of the sterols fraction	69
Table (5) Retention times and relative percentage of the fatty acids fraction from GLC	73
Table (6) Rf values of fraction (A)	91
Table (7) GC/MS conditions and column	103
Table (8) Physical signs and qualitative manifestations of acute toxicity tests in rodents	128
Table (9) Post-mortem evidence of lethal toxic overdosage detectable by naked eye examination	130
Table (10) Acute i.p. lethal toxicity of total ethanolic extract	144
Table (11) Acute i.p. lethal toxicity of defatted ethanolic extract	145
Table (12) Test of parallelism of acute i.p. log dose mortality lines of total and defatted ethanolic extracts	147
Table (13) Comparative behavioural feature of i.p. versus oral toxic overdosage of the oil fraction	149
Table (14a) Comparative prophylactic efficacies of the isolated fractions against ovalbumin induced	

paw-oedema swelling	164
Table (14b) Comparative effects of one week medication on	
body weight gains	165
Table (15) Non-paired student's "t" test for the body	
weight gains	166
Table (16) Comparative prophylactic efficacies of one week	
medication against lethal anaphylactic	
reactions	167

LIST OF FIGURES

	PAGE
Chart (1)	37
Scheme (1-5)	50,51
(Fig 1) GLC chromatogram of standard hydrocarbons and sterols	67
(Fig 2) GLC chromatogram of the hydrocarbons and sterols fraction	68
(Fig 3) GLC chromatogram of the total fatty acids	74
(Fig 4-12) GC/MS of the total fatty acids	77-85
(Fig 13) TLC chromatogram of fraction (B)	89
(Fig 14) TLC chromatogram of fraction (A)	92
(Fig 15) Ultra-violet spectrum of fraction (A)	95
(Fig 16) Infra-red spectrum of Aa	96
(Fig 17) Infra-red spectrum of coumarin (Aldrich)	96
(Fig 18) Infra-red spectrum of Ab	97
(Fig 19) Infra-red spectrum of 7-methoxycoumarin (Aldrich)	97
(Fig 20) HPLC chromatogram of fraction (A)	99
(Fig 21) HPLC chromatogram of standard herniarin	100
(Fig 22) HPLC chromatogram of standard coumarin	101
(Fig 23i,ii) GC/MS chromatogram of coumarin (i) and 7-methoxycoumarin (ii)	104
(Fig 24) Ultra-violet spectrum of fraction (B)	106
(Fig 25) Infra-red spectrum of fraction (B)	107
(Fig 26) EI mass spectrum of fraction (B)	110
(Fig 27) CI mass spectrum of fraction (B)	110
Scheme (6)	111

(Fig 28) NMR spectrum of fraction (B)	114
Scheme (7)	117
(Fig 30) TLC chromatogram of the aglycone of fraction (B).....	118
(Fig 31) Paper chromatography of fraction (B) hydrolyzate	119
(Fig 32) Best-fitting acute i.p.dose mortality line of total and defatted ethanolic extracts	146
(Fig 33) Bar-chart showing comparative magnitudes of three conventional acute i.p. lethal doses of total and defatted ethanolic extracts	148
(Fig 34) Bar-chart showing comparative magnitudes and statistical significance of mean percentage estimates of paw-oedema swelling	168

Summary

The research investigations presented in this thesis deal with chemical studies of *Prunus mahaleb* L. kernels besides pharmacotoxicity screening of certain organic extracts and the oil fraction isolated therefrom.

The text of this thesis includes:

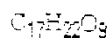
1- Comprehensive review of published reports in literature on the chemical constituents of *Prunus mahaleb* L. kernels notably with reference to coumarins and lipid fraction contents.

2- The phytochemical study of the coumarins fraction resulted in the isolation of three compounds; one of them isolated for the first time.

These compounds were identified by:

UV, IR, MS, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$.

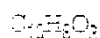
* The new compound: 2-glucosyloxy-4-methoxy methyl *trans* cinnamate



* Coumarin



and * 7-methoxy coumarin



3- The fractionation of the oil resulted in the isolation and identification of the following by gas chromatography and gas chromatography/ mass spectrometry techniques:-

a) The fatty acids which indicated the presence of 27 fatty acids, 17 of them were identified;

from which oleic acid (C_{18}) and linoleic acid (C_{18}) were the major constituents.

b) The hydrocarbons fraction resulted in the isolation and identification of 18 hydrocarbons ($n-C_{10}$ - $n-C_{22}$)

c) The sterols fraction comprising:-

- 1- Cholesterol
- 2- Stigmasterol
- 3- β -Sitosterol

and 4- Campesterol

4- The mucilage fraction represented the presence of only one sugar which was identified as Arabinose.

5- The pharmacotoxicity studies were carried out on the total and defatted ethanolic extracts as well as the oil fraction:-

* The acute toxicity determinations were undertaken to define the limits of the lethal toxic doses; using the method of Litchfield and Wilcoxon for statistical evaluation of dose mortality data yielding the following estimates:-

	i.p.LD ₅₀	i.p.LD ₅₀	i.p.LD ₅₀
Total extract	5	7	8
Defatted extract	3.25	4.5	6

The oil fraction proved to be extremely safe and free from any acute lethal toxicity in graded i.p. and oral doses up to 100ml/kg B.W.

* In-vivo assessment of antiproliferative efficiency afforded by 7 days course of daily medication