

# بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ



HOSSAM MAGHRABY



# شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HOSSAM MAGHRABY

# جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم  
قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HOSSAM MAGHRABY



بعض الوثائق

الأصلية تالفة



HOSSAM MAGHRABY



بالرسالة صفحات

لم ترد بالأصل



HOSSAM MAGHRABY

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**PHYTOCHEMICAL INVESTIGATION OF  
CERTAIN *ASTRAGALUS* SPECIES  
GROWN IN EGYPT**

BIKAE7

Thesis Presented  
By

**Mohamed Mahmoud Ahmed Radwan**

(B. Pharm. Sci. 1994)  
University of Alexandria

In The Partial Fulfillment For The Degree Of  
Master Of Pharmaceutical Science  
(Pharmacognosy)

**Department of Pharmacognosy  
Faculty of Pharmacy  
University of Alexandria  
Egypt.**

**2000**

# Supervisory Committee

Prof. Dr.

**Nadia A. El-Sebakhy**

Professor of Pharmacognosy  
Faculty of Pharmacy  
University of Alexandria

Dr.

**Aya M. Asaad**

Assistant Professor of Pharmacognosy  
Faculty of Pharmacy  
University of Alexandria

Dr.

**Soad M. Toaima**

Lecturer of Pharmacognosy  
Faculty of Pharmacy  
University of Alexandria

# CONTENTS

Page

LIST OF TABLES .....	i
LIST OF FIGURES.....	iii
ABBREVIATIONS.....	v
INTRODUCTION .....	1
AIM OF THE WORK.....	57
MATERIALS, REAGENTS AND APPARATUS .....	60
PHYTOCHEMICAL SCREENING OF <i>ASTRAGALUS</i> <i>KAHIRICUS</i> DC. AND <i>ASTRAGALUS HAMOSUS</i> L. ....	64

## PART I

### PHYTOCHEMICAL STUDY OF THE ROOTS OF *ASTRAGALUS KAHIRICUS* DC.

#### CHAPTER I: ISOLATION OF FIVE MATERIALS FROM THE ROOTS OF *A. KAHIRICUS* DC.

A- Fractionation of the methylene chloride extract and characterization of material <b>K1</b> .....	65
B- Fractionation of the ethyl acetate extract.....	68
C- Fractionation of the n-butanol extract.....	69

#### CHAPTER II: CHARACTERIZATION OF THE ISOLATED MATERIALS FROM THE ROOTS OF *A. KAHIRICUS* DC.

Characterization of saponin <b>K2</b> .....	70
Characterization of saponin <b>K3</b> .....	94
Characterization of saponin <b>K4</b> .....	103
Characterization of saponin <b>K5</b> .....	111

## PART II

### PHYTOCHEMICAL STUDY OF *ASTRAGALUS HAMOSUS* L.

#### CHAPTER I: ISOLATION OF FOUR MATERIALS FROM *ASTRAGALUS HAMOSUS* L. Page

- A- Fractionation of the methylene chloride extract and characterization  
of material **H1** and material **H2**(still under investigation)..... 120
- B- Fractionation of the ethyl acetate extract.....123

#### CHAPTER II: CHARACTERIZATION OF THE ISOLATED MATERIALS FROM *ASTRAGALUS HAMOSUS*

- Characterization of flavone **H3**..... 124
- Characterization of saponin **H4**..... 133

## PART III

#### IMMUNOSTIMULANT ACTIVITY OF SOME ISOLATED CYCLOARTANE SAPONINS .....143

#### GENERAL SUMMARY AND CONCLUSION .....145

#### REFERENCES .....148

#### ARABIC SUMMARY

## LIST OF TABLES

	page
Table I : Cycloartane saponins isolated from genus <i>Astragalus</i> ...	4
Table II : Isolated oleanene-type saponins from genus <i>Astragalus</i> ...	22
Table III : Flavonols of genus <i>Astragalus</i> .....	26
Table IV : Isoflavans of genus <i>Astragalus</i> .....	31
Table V : Isoflavones of genus <i>Astragalus</i> .....	33
Table VI : Flavones of genus <i>Astragalus</i> .....	34
Table VII : Flavanones of genus <i>Astragalus</i> .....	34
Table VIII : Pterocarpanes of genus <i>Astragalus</i> .....	35
Table IX : Isolated Alkaloids from genus <i>Astragalus</i> .....	37
Table X : Screening of <i>A. kahiricus</i> DC. and <i>A. hamosus</i> L. extracts..	64
Table XI : Fractionation of the methylene chloride extract of <i>A. kahiricus</i> DC. roots.....	66
Table XII : Fractionation of the ethyl acetate extract of <i>A. kahiricus</i> DC. roots.....	68
Table XIII : Fractionation of the n-butanol extract of <i>A. kahiricus</i> DC. roots.....	69
Table XIV : <sup>13</sup> CNMR data and C-H connectivities as determined by HMBC of <b>K2</b> in comparison with <sup>13</sup> CNMR <b>cycloastragenol</b>	72
Table XV : <sup>1</sup> H and <sup>13</sup> CNMR assignments of sugar moieties of <b>K2</b> as determined from HMQC and HMBC experiments.....	73
Table XVI : <sup>13</sup> CNMR data of saponin <b>K3</b> in comparison with <b>astrasieversianin VI</b> and <b>cycloastragenol</b> .....	96
Table XVII : <sup>13</sup> CNMR data of saponin <b>K4</b> in comparison with <b>astrasieversianin X</b> and <b>cycloastragenol</b> .....	105

<b>Table XVIII</b>	: $^{13}\text{C}$ NMR data of saponin <b>K5 methyl ester</b> in comparison with <b>wistariasaponin C methyl ester</b> and <b>soyasapogenol B</b>	113
<b>Table XIX</b>	: Fractionation of the methylene chloride extract of <i>A.hamosus</i> L.....	121
<b>Table XX</b>	: UV spectral data of <b>H3</b> .....	126
<b>Table XXI</b>	: $^{13}\text{C}$ NMR data of flavone <b>H3</b> in comparison with <b>thetiaflavone</b> .....	127
<b>Table XXII</b>	: $^{13}\text{C}$ NMR data of saponin <b>H4</b> in comparison with <b>astraverrucin VI</b> and <b>cycloastragenol</b> .....	135
<b>Table XXIII</b>	: $^1\text{H}$ NMR and $^{13}\text{C}$ NMR assignments of the sugar moieties of saponin <b>H4</b> .....	136
<b>Table XXIV</b>	: Effect of saponins <b>K3</b> , <b>K4</b> , and <b>H4</b> on lymphocyte transformation with Con. A(8 ug) treated mouse lymphocytes	144

## LIST OF FIGURES

	page
Figure 1: photograph of <i>Astragalus kahiricus</i> DC.....	59
Figure 2: photograph of <i>Astragalus hamosus</i> L.....	59
Figure 3 : IR spectrum of saponin <b>K2</b> .....	74
Figure 4 : FAB mass of saponin <b>K2</b> .....	75
Figure 5 : <sup>1</sup> HNMR spectra of saponin <b>K2</b> .....	76-78
Figure 6 : <sup>13</sup> CNMR spectra of saponin <b>K2</b> .....	79-82
Figure 7 : HMBC spectra of saponin <b>K2</b> .....	83-85
Figure 8 : HMBC of saponin <b>K2</b> showing linkages between protons and carbons.....	86
Figure 9 : <sup>1</sup> H, <sup>1</sup> H-COSY spectra of saponin <b>K2</b> .....	87-88
Figure 10 : 2D TOCSY(HOHAHA) spectrum of saponin <b>K2</b> .....	89
Figure 11 : 2D TOCSY(HOHAHA) of saponin <b>K2</b> showing H-H connectivities of the sugar moieties .....	90
Figure 12 : IR spectrum of saponin <b>K3</b> .....	97
Figure 13 : FAB mass of saponin <b>K3</b> .....	98
Figure 14 : <sup>1</sup> HNMR spectrum of saponin <b>K3</b> .....	99
Figure 15 : <sup>13</sup> CNMR spectrum of saponin <b>K3</b> .....	100
Figure 16 : FAB mass of saponin <b>K4</b> .....	106
Figure 17 : <sup>1</sup> HNMR spectrum of saponin <b>K4</b> .....	107
Figure 18 : <sup>13</sup> CNMR spectrum of saponin <b>K4</b> .....	108
Figure 19 : IR spectrum of saponin <b>K5</b> .....	114
Figure 20 : <sup>1</sup> HNMR spectrum of saponin <b>K5 Methyl ester</b> .....	115
Figure 21 : <sup>13</sup> CNMR spectra of saponin <b>K5 Methyl ester</b> .....	116-117

<b>Figure 22</b> : UV spectra of flavone <b>H3</b> .....	126
<b>Figure 23</b> : UV spectrum of the aglycone of <b>H3</b> (in methanol).....	126
<b>Figure 24</b> : Electrospray MS of flavone <b>H3</b> .....	128
<b>Figure 25</b> : $^1\text{H}$ NMR spectrum of flavone <b>H3</b> .....	129
<b>Figure 26</b> : $^{13}\text{C}$ NMR spectrum of flavone <b>H3</b> .....	130
<b>Figure 27</b> : FAB mass of saponin <b>H4</b> .....	137
<b>Figure 28</b> : $^1\text{H}$ NMR spectrum of saponin <b>H4</b> .....	138
<b>Figure 29</b> : $^{13}\text{C}$ NMR spectrum of saponin <b>H4</b> .....	139
<b>Figure 30</b> : HMBC spectrum of saponin <b>H4</b> .....	140

## ABBREVIATIONS

<b>A.</b>	: Astragalus
<b>#</b>	: Structure number
<b>ref.</b>	: Reference number
<b>muli.</b>	: Multiplicity
<b>undiff.</b>	: Undifferentiated
<b>xyl</b>	: Xylose
<b>glc</b>	: Glucose
<b>rhm</b>	: Rhamnose
<b>gal</b>	: Galactose
<b>api</b>	: Apiofructose
<b>glcUA</b>	: Glucuronic acid
<b>ara</b>	: Arabinose
<b>rob</b>	: Robinose
<b>rut</b>	: Rutinose
<b>gent</b>	: Gentiobiose
<b>OAc</b>	: Acetate
<b>WHO</b>	: World Health Organization
<b>NK</b>	: Natural Killer
<b>INF</b>	: Interferon
<b>HIV</b>	: Human Immune Deficiency Virus
<b>TPA</b>	: Tetradecanyl phorbol acetate
<b>TNF</b>	: Tumor Necrosing Factor
<b>SGPT</b>	: Serum Glutamic Pyruvate Transaminase
<b>EAC</b>	: Ehrlich ascities Carcinoma
<b>ATP</b>	: Adenosine Triphosphate
<b>TLC</b>	: Thin Layer Chromatography
<b>PTLC</b>	: Preparative Thin Layer Chromatography
<b>Con. A</b>	: Concanavalin A
<b>Me</b>	: Methyl
<b>C<sub>5</sub>D<sub>5</sub>N</b>	: Deuterated pyridine
<b>CDCl<sub>3</sub></b>	: Deuterated chloroform
<b>DMF</b>	: Dimethylformamide
<b>DMSO</b>	: Dimethylsulphoxide
<b>But</b>	: Butenoyl