

# **Phytochemical and Biological Studies on** ***Albizia anthelmintica* Family Fabaceae**

A Thesis Submitted  
By

**Dina Mohamed Bahgat Mohamed Saed Seif**

Bachelor of Pharmaceutical Sciences,  
Faculty of Pharmacy, Ain Shams University, 2009

In Partial Fulfilment of the Requirements  
For the Degree of Masters in Pharmaceutical Sciences  
(Pharmacognosy)

**Under the Supervision of**

**Abdel-Nasser B. Singab, Ph.D.**

Professor of Pharmacognosy  
Dean of Faculty of Pharmacy  
Ain Shams University  
Health Minister Consultant for Pharmacy Affairs

**Omayma A. Eldahshan, Ph.D.**

Associate Professor of Pharmacognosy  
Acting Head of Pharmacognosy Department  
Faculty of pharmacy  
Ain Shams University

**Eman Kamal Al-Sayed, Ph.D.**

Lecturer of Pharmacognosy  
Faculty of pharmacy  
Ain Shams University

Department of Pharmacognosy  
Faculty of Pharmacy  
Ain Shams University  
Abbassia, Cairo, Egypt  
2016

*I dedicate this work to the soul of*

*My mother Aziza*

*Who taught me the meaning of hard work, how to be  
Self-dependent and patient, without her prayers and love*

*I wouldn't have achieved anything in my life*

# Acknowledgement

First of all, I would like to extend due praise and thanks to **ALLAH**, the source of all knowledge, and may His peace and blessings be upon all his prophets; for helping and guiding me throughout my life.

I would like to express my deepest gratitude, sincere and profound appreciation to the following people who significantly contributed to the work done in this thesis:

Members of the advisory committee; **Prof. Dr. Abdel-Nasser B. Singab**, Professor of Pharmacognosy, Dean of Faculty of Pharmacy, Ain Shams University, for his advice, constant guidance, helpful suggestions and encouragement throughout this work. Thanks for his precious time, his support, sincere comments, and great efforts in revising the thesis. Thanks for setting an example to what a dedicated professor, scientist and advisor should be.

I am deeply grateful to **Assoc. Prof. Dr. Omayma A. Eldahshan**, Associate Professor and acting head of Pharmacognosy department, Faculty of Pharmacy, Ain Shams University, for suggesting the research point, providing valuable comments, encouragement during the course of this work, for helping me in the structure elucidation of the compounds, her patience, time and great efforts in revising the thesis and most of all her continuous support to me for which I am truly grateful. **Dr. Eman Kamal Al-Sayed**, lecturer of Pharmacognosy, Faculty of pharmacy, Ain Shams University for her time and patience in teaching me, in the structure elucidation of the compounds and for her extensive guidance in revising the thesis. The long hours I spent with **both of them** in the lab., where they sincerely taught me and transferred to me their knowledge and experience, they supported and encouraged me in every step, I am indebted to them with every single word in this thesis as I am lucky to be their student. Telling them thanks for being serious, committed and so patient through this work.

I am grateful to the Science and Technology Development Fund in Egypt STDF (drug discovery and development center) for its grant N-5251 that gave me access for NMR measurements. I am also thankful to Dr. Maarit Karonen, Department of Chemistry, University of Turku Finland for carrying out HRESIMS measurements.

My doctors in the department of Pharmacognosy; I am very thankful for every one of them for their continuous advising, support and encouragement. Especially **Dr. Sherif Ebada**, **Dr. Mohamed El-Shazly** and **Dr. Mohamed Ashour** for their help in searching Scifinder for the new compounds.

My colleagues; for their cooperation, support and the friendship we share, especially those who spent with me long and hard times in lab; **Nouran**, **Heba**, **Esraa**, and **Shaimaa**. **Dr. Ahmed Essam** who was responsible for NMR measurements and **Dr. Mohammed Saed** for his sincere and valuable advice.

I would like to thank **my dearest great father whom I really love and respect**, for his care, love, constructive advice, guidance and continuous prayers, I am proud to be his daughter and **my brothers**, **Haitham** and **Yassin** and **my little sister Hadeer** for their continuous care, support and love which always encouraged me to do my best; thank you very much really I love you so much. Also I thank my grandfather **Prof. Dr. Abdel Fattah Khodeir**, and my uncle **Assoc. Prof. Dr. Taha Khodeir** for being my role model, my mother in law for helping me and for her sincere prayers.

Finally, I would like to thank my dear husband, **Ahmed** for his continuous care, love, support, motivation, sincere advice and most of all his patience for which I am truly grateful. His continuous encouragement really pushed me forward to do my best. My little princess **Rodaina**, the joy of my life, her shiny smile made me pass the hard times.

*Dina Mohammed Bahagt Seif*

**Cairo, 2016**

## List of Contents

	<b>Page</b>
<b>Contents</b> .....	i
<b>List of Figures</b> .....	ii
<b>List of Tables</b> .....	v
<b>List of Abbreviations</b> .....	vi
<b>Introduction</b> .....	1
<b>Review of Literature</b> .....	4
1-Folk Medicinal Uses of <i>Albizia</i> Species.....	4
2-Chemical Review of Genus <i>Albizia</i> .....	7
3-Biological Review of Genus <i>Albizia</i> .....	35
<b>Taxonomy</b> .....	46
<b>Materials, Apparatus and Methods</b> .....	52
<b>Chapter 1: Biological Investigation of the 80% Aqueous Methanol Extract of <i>Albizia anthelmintica</i> Brongn. Leaves Family Fabaceae</b> .....	61
1- Determination of Cytotoxic Activity.....	61
2- Determination of Molluscicidal Activity.....	78
<b>Chapter 2: Phytochemical Investigation of the 80% Aqueous Methanol Extract of <i>Albizia anthelmintica</i> Brongn. Leaves Family Fabaceae</b> .....	81
1. Preliminary Phytochemical Screening.....	81
2. Fractionation of the 80% Aqueous Methanol Extract of <i>A. anthelmintica</i> Brongn. Leaves	82
Identification of Compound <b>1</b> .....	85
Identification of Compound <b>2</b> .....	89
Identification of Compound <b>3</b> .....	101
Identification of Compound <b>4</b> .....	113
Identification of Compound <b>5</b> .....	124
<b>General Summary</b> .....	134
<b>Conclusion and Recommendations</b> .....	138
<b>References</b> .....	139
<b>Arabic Summary</b> .....	

## List of Figures

Figure No.	Page
1. Structures of saponins isolated from genus <i>Albizia</i> .....	12
2. Structures of alkaloids isolated from genus <i>Albizia</i> .....	24
3. Structures of flavonoids isolated from genus <i>Albizia</i> .....	29
4. Structures of phenolic glycosides and phenolic acids isolated from genus <i>Albizia</i> .....	33
5. Geographical distribution of <i>A. anthelmintica</i> .....	49
6. <i>A. anthelmintica</i> tree.....	50
7. <i>A. anthelmintica</i> flower.....	50
8. <i>A. anthelmintica</i> leaves.....	51
9. <i>A. anthelmintica</i> pods.....	51
10. Cytotoxic effect of acetone soluble fraction on HepG 2.....	66
11. Cytotoxic effect of acetone insoluble fraction on HepG 2.....	66
12. Cytotoxic effect of fraction I on HepG 2.....	67
13. Cytotoxic effect of fraction II on HepG 2.....	67
14. Cytotoxic effect of fraction III on HepG 2.....	68
15. Cytotoxic effect of fraction IV on HepG 2.....	68
16. Cytotoxic effect of saponin <b>2</b> on HepG 2.....	69
17. Cytotoxic effect of saponin <b>3</b> on HepG 2.....	69
18. Cytotoxic effect of saponin <b>4</b> on HepG 2.....	70
19. Cytotoxic effect of saponin <b>5</b> on HepG 2.....	70
20. Cytotoxic effect of doxorubicin on HepG 2 .....	71
21. Cytotoxic effect of doxorubicin on HCT-116.....	71
22. Cytotoxic effect of acetone soluble fraction on HCT-116.....	72
23. Cytotoxic effect of acetone insoluble fraction on HCT-116.....	72
24. Cytotoxic effect of fraction I on HCT-116.....	73
25. Cytotoxic effect of fraction II on HCT-116.....	73
26. Cytotoxic effect of fraction III on HCT-116.....	74
27. Cytotoxic effect of fraction IV on HCT-116.....	74

28. Cytotoxic effect of saponin <b>2</b> on HCT-116.....	75
29. Cytotoxic effect of saponin <b>3</b> on HCT-116.....	75
30. Cytotoxic effect of saponin <b>4</b> on HCT-116.....	76
31. Cytotoxic effect of saponin <b>5</b> on HCT-116.....	76
32. General fractionation scheme.....	83
33. Structure of compound <b>1</b> : kampferol-3- <i>O</i> - $\beta$ -D-glucopyranoside	86
34. $^1\text{H}$ -NMR of compound <b>1</b> (DMSO- $d_6$ , full spectrum).....	87
35. APT of compound <b>1</b> (DMSO- $d_6$ , full spectrum).....	88
36. Structure of compound <b>2</b> : 3- <i>O</i> -[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl]-28- <i>O</i> -[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl] oleanolate.....	91
37. $^1\text{H}$ NMR spectrum of compound <b>2</b> (CD $_3$ OD, full spectrum).....	94
38. APT spectrum of compound <b>2</b> (CD $_3$ OD, full spectrum).....	95
39. APT spectrum of compound <b>2</b> (CD $_3$ OD, aglycone region).....	96
40. APT spectrum of compound <b>2</b> (CD $_3$ OD, sugar region).....	97
41. HSQC correlations of compound <b>2</b> (anomeric carbons and protons).....	98
42. HMBC and COSY correlations of compound <b>2</b> .....	99
43. (a). HRESIMS of compound <b>2</b> (negative mode) at 31.5-38.5 eV.....	100
43. (b). HRESIMS of compound <b>2</b> (negative mode) at 67.5- 82.5 eV.....	100
43. (c). Fragmentation pattern of compound <b>2</b> .....	100
44. Structure of compound <b>3</b> : 3- <i>O</i> -[ $\beta$ -D-Xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl]-28- $\beta$ -D-glucopyranosyl oleanolate.....	102
45. $^1\text{H}$ NMR spectrum of compound <b>3</b> (CD $_3$ OD, full spectrum).....	105
46. APT spectrum of compound <b>3</b> (CD $_3$ OD, full spectrum).....	106
47. APT spectrum of compound <b>3</b> (CD $_3$ OD, aglycone region).....	107
48. APT spectrum of compound <b>3</b> (CD $_3$ OD, expanded spectrum).....	108
49. APT spectrum of compound <b>3</b> (CD $_3$ OD, sugar region).....	109
50. HSQC correlations of compound <b>3</b> (anomeric carbons and protons)...	110
51. HMBC and COSY correlations of compound <b>3</b> .....	111
52. (a). HRESIMS of compound <b>3</b> (negative mode) 4.5-5.5 eV.....	112

52. (b). HRESIMS of compound <b>3</b> (negative mode) 27-33 eV.....	112
52. (c). Fragmentation pattern of compound <b>3</b> .....	112
53. Structure of compound <b>4</b> : 3- <i>O</i> -[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl] oleanolic acid.....	114
54. $^1\text{H}$ -NMR of compound <b>4</b> (DMSO- $d_6$ , full spectrum).....	117
55. APT of compound <b>4</b> (DMSO- $d_6$ , full spectrum).....	118
56. APT of compound <b>4</b> (DMSO- $d_6$ , sugar region).....	119
57. APT of compound <b>4</b> (DMSO- $d_6$ , aglycone region).....	120
58. HSQC correlations of compound <b>4</b> (anomeric carbons and protons)...	121
59. HMBC and COSY correlations of compound <b>4</b> .....	122
60. (a). HRESIMS of compound <b>4</b> (negative mode) 22.5-27 eV.....	123
60. (b). HRESIMS of compound <b>4</b> (negative mode) 27-33 eV.....	123
60. (c). Fragmentation pattern of compound <b>4</b> .....	123
61. Structure of compound <b>5</b> : 3- <i>O</i> -[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranosyl] oleanolic acid.....	125
62. $^1\text{H}$ NMR spectrum of compound <b>5</b> (DMSO- $d_6$ , full spectrum).....	127
63. APT spectrum of compound <b>5</b> (DMSO- $d_6$ , full spectrum).....	128
64. APT spectrum of compound <b>5</b> (DMSO- $d_6$ , aglycone region).....	129
65. APT spectrum of compound <b>5</b> (DMSO- $d_6$ , sugar region).....	130
66. HSQC correlations of compound <b>5</b> (anomeric carbon and protons).....	131
67. HMBC and COSY correlations of compound <b>5</b> .....	132
68. (a). HRESIMS of compound <b>5</b> (negative mode) 4.5-5.5 eV.....	133
68. (b). HRESIMS of compound <b>5</b> (negative mode) 35-44 eV.....	133
68. (c). Fragmentation pattern of compound <b>5</b> .....	133
69. Fractionation scheme of the extract and isolation of compounds.....	137



## List of Tables

Table No.	Page
1- Distribution of saponins in genus <i>Albizia</i> .....	7
2- Distribution of alkaloids in genus <i>Albizia</i> .....	23
3- Distribution of phenolic compounds and flavonoids in genus <i>Albizia</i> .....	27
4- Cytotoxic activity of saponins isolated from different <i>Albizia</i> species.....	36
5- Taxonomical classification of <i>Albizia anthelmintica</i> .....	49
6- The cell viability of HepG 2 treated with acetone sol. and acetone insol. fractions, sub-fractions I-IV and the pure saponins <b>2-5</b> .....	64
7-The cell viability of HCT-116 treated with acetone sol. and acetone insol. fractions, sub-fractions I-IV and the pure saponins <b>2-5</b> .....	65
8- Molluscicidal activity against <i>Biomphalaria alexandrina</i> snails.....	79
9- The percentage mortality of <i>S. mansoni</i> miracidia.....	79
10- The percentage mortality of <i>S. mansoni</i> cercaria.....	79
11- Phytochemical screening of <i>A. anthelmintica</i> leaves.....	81
12- Sub-Fractions of Column No. 1.....	83
13- Sub-Fractions of Column No. 2.....	84
14- Spectral data of compound <b>1</b> .....	86
15- Sub-Fractions of Column No. 4.....	89
16- $^1\text{H}$ and $^{13}\text{C}$ chemical shifts of compound <b>2</b> and HMBC correlations.....	92
17- $^1\text{H}$ and $^{13}\text{C}$ chemical shifts of compound <b>3</b> and HMBC correlations.....	103
18- $^1\text{H}$ and $^{13}\text{C}$ chemical shifts of compound <b>4</b> and HMBC correlations .....	115
19- $^1\text{H}$ and $^{13}\text{C}$ chemical shifts of compound <b>5</b> and HMBC correlations.....	125
20-Summary of the isolated compounds.....	136

## **List of Abbreviations**

<b>ALP</b>	Alkaline Phosphatase
<b>ALT</b>	Alkaline Transaminase
<b>APT</b>	Attached Proton Test
<b>Ara</b>	Arabinose
<b>AST</b>	Aspartate Transaminase
<b>A2780</b>	Human Ovarian Carcinoma Cell Line
<b>A549</b>	Human Lung Epithelial Carcinoma Cell Line
<b><i>brs</i></b>	Broad singlet
<b>BEL 7402</b>	Hepatocellular Carcinoma Cell Line
<b>BGC 823</b>	Human Gastric Cancer Cell Line
<b>CC</b>	Column Chromatography
<b><math>^{13}\text{C}</math>-NMR</b>	Carbon-13 Nuclear Magnetic Resonance
<b><math>\text{CD}_3\text{OD}</math></b>	Deutrated methanol
<b>conc.</b>	Concentrated
<b>COSY</b>	Correlation Spectroscopy
<b><i>d</i></b>	Doublet
<b><i>dd</i></b>	Doublet of doublet
<b>dil.</b>	Diluted
<b><math>\text{DMSO-}d_6</math></b>	Deutrated Dimethyl Sulfoxide
<b>ELISA</b>	Enzyme-Linked Immuno-Sorbent Assay
<b>EDTA</b>	Ethylene Diamine Tetra Acetic acid
<b>FBS</b>	Fetal Bovine Serum
<b>Fig.</b>	Figure
<b>GGT</b>	Gamma Glutamyl Transferase
<b>Glc</b>	Glucose
<b><math>^1\text{H}</math>-NMR</b>	Proton Nuclear Magnetic Resonance

<b>HCT-8, HCT-116</b>	Human Colon Carcinoma Cell Line
<b>HT-29</b>	Human Colorectal Adenocarcinoma Cell Line
<b>HepG-2</b>	Human Hepatocellular Carcinoma Cell Line
<b>HMBC</b>	Heteronuclear Multiple Bond Correlation
<b>HPLC</b>	High Performance Liquid Chromatography
<b>HRESIMS</b>	High Resolution ElectroSpray Ionization Mass Spectrometry
<b>HSQC</b>	Heteronuclear Single Quantum Correlation
<b>Hz</b>	Hertz
<b>IC<sub>50</sub></b>	Inhibitory Concentration showing 50 % inhibition
<b><i>J</i> value</b>	Coupling Constant
<b>KB</b>	Oral Carcinoma Cell Line
<b>LC<sub>50</sub></b>	Lethal Concentration to 50%
<b>LC<sub>90</sub></b>	Lethal Concentration to 90%
<b>LD<sub>50</sub></b>	Lethal Dose showing 50% inhibition
<b>LED</b>	Light Emitting Diode
<b><i>m</i></b>	multiplet
<b>MCF 7</b>	Human Breast Adenocarcinoma Cell Line
<b>mp</b>	Melting point
<b>MTT</b>	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
<b>MS</b>	Mass Spectrometry
<b><i>m/z</i></b>	Mass to charge ratio
<b>OA</b>	Oleanolic Acid
<b>OD</b>	Optical Density
<b>ppm</b>	Part per million
<b>RPMI</b>	Roswell Park Memorial Institute medium
<b>R<sub>f</sub></b>	Retardation factor
<b>Rha</b>	Rhamnose
<b><i>s</i></b>	Singlet

<b>SRB</b>	Sulphorhodamine B
<b>TLC</b>	Thin Layer Chromatography
<b>TMS</b>	Tetramethylsilane
<b>Xyl</b>	Xylose
<b>µg/mL</b>	Microgram per milliliter
<b>µM</b>	Micromole
<b>UV</b>	Ultraviolet
<b>δ</b>	Chemical shift by delta value
<b>λ</b>	Wave length

# Introduction

Medicinal plants are considered a treasure house of potential drugs and recently there has been an increasing awareness about their importance. This interest in drugs of natural origin is due to several reasons, including the frequent inefficiency of conventional medicine, possible development of side effects of synthetic drugs and also large percentage of the world's poor population doesn't have access to these synthetic drugs. Furthermore, the long history of use of folk medicine suggests that natural products have fewer side effects compared to synthetic drugs. It has been estimated that in developed countries such as the United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80% [Kokila *et al.*, 2013]. The plant kingdom offers a unique and renewable resource for the discovery of potential new drugs and important lead compounds against various pharmacological targets including pain, cancer, HIV, Alzheimer's disease and malaria [Farag *et al.*, 2013].

In Egypt, many plants have been used in folk medicine. The ancient Egyptians were familiar with many medicinal herbs and aware of their usefulness in the treatment of various diseases. They used the plant organs such as the roots, rhizomes, flowers, leaves, fruits and seeds. They applied their medicaments in the form of powders, pills, suppositories, creams, pastes and ointments. However, scientific evidence for the medicinal properties of such plants was not always demonstrated [Mohamed *et al.*, 2013].

Family Fabaceae (legume or bean family) is a large and economically important family of flowering plants. It includes trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound stipulated leaves. The family is widely distributed and is the third-largest land plant family in terms of number of species [Stevens, 2006]. Legumes are economically and culturally important plants due to their extraordinary diversity and abundance, they comprise a wide variety of edible vegetables that contain oil and fats with different uses. Their medicinal value is due to their effectiveness in the treatment of a wide variety of human diseases. The diversity of chemically active constituents, such as tannins, flavonoids, alkaloids and terpenes found in the plants of this family are known to have a potent biological activity [Molares and Ladio, 2011]

*Albizia* is a large genus belonging to the family Fabaceae, which comprises about 150 species that are widely distributed in the tropics, with the great diversity in Africa and Central South America. They are commonly called silk plants or silk trees. The generic name honors the Italian nobleman Filippo degli Albizzi, who introduced *Albizia julibrissin* to Europe in the mid-18<sup>th</sup> century. Albizias are important forage, timber, medicinal plants and many are cultivated as ornamentals for their attractive flowers. *Albizia* species have been reported to be rich in phenolic compounds, steroidal and triterpenoidal saponins [Joycharat *et al.*, 2013]. *Albizia*, Tree of Happiness is used in traditional Chinese medicine for relieving depression, insomnia, irritability and stress. [Chen and Chen, 2001]. It is also used by native tribes in tropical and subtropical regions of Asia and Africa for the treatment of skin problems, malaria, cough, dysentery, amoebiasis and as an anthelmintic [Kokila *et al.*, 2013].

*Albizia anthelmintica* Brong. or worm cure *Albizia* is a bush that is common in western part of Sudan and also cultivated in Egypt. It is an attractive tree in bloom, with fluffy-cream coloured, scented flowers and is suitable for ornamental purposes. Its wood is used for manufacturing furniture. The stem bark has been used in Sudanese folk medicine for the treatment of tape worm infection, stomach troubles, amoebic dysentery and less frequently for malaria [Broun and Massey, 1929]. The plant is widely used in East Africa by small holder farmers to treat their livestock against internal parasites [Anon, 1996].

**The main objective of this study** was to investigate the cytotoxic bioactivity of *A. anthelmintica* leaves and to determine its phytochemical composition, in order to identify the compounds responsible for this bioactivity. The selected plant leaves was chosen based on preliminary phytochemical screening which revealed the presence of saponins and phenolic compounds. Though many phytochemical constituents and promising pharmacological activities were reported from different plants belonging to this genus, yet nothing could be traced concerning the saponin content of *A. anthelmintica*.

In addition, few studies were traced in the literature on the pharmacological activity of *A. anthelmintica*, mainly about the anthelmintic activity of the bark [Koko *et al.*, 2000; Gradé *et al.*, 2008], and the antioxidant activity of the leaves [Mohamed *et al.*, 2013]. Therefore, it was found interesting to carry out another perspective of biological and phytochemical investigation on *A. anthelmintica* leaves.

**The steps of the protocol were done as follows:**

1. Collection, identification and drying of plant material.
2. Phytochemical screening of the plant.
3. Preparation of the plant extract.
4. Fractionation of the plant extract using various chromatographic techniques.
5. Determination of the biological activity of the extract, fractions and isolated compounds.
6. A phytochemical study of the most biologically active fraction using chromatographic techniques to isolate the compounds responsible for the bioactivity.
7. Structural identification of the isolated compounds using spectroscopic and spectrometric techniques.