



**PHARMACOGNOSTICAL AND BIOLOGICAL  
STUDIES OF *PLUMBAGO AURICULATA* Lam.**

**A Thesis**

**Submitted for the Degree of Doctor of  
Philosophy in Botany**

**By**

**Adawia Mahmoud Kitaz  
{M. Sc.}**

**Under Supervision**

**Dr. Magdy Mohamed Mourad**

*Ass. Prof. of Plant Taxonomy  
Faculty of Science, Ain -Shams University*

**Dr. Fatma Abdel Kader Moharram**

*Ass. Prof. of Pharmacognosy  
Faculty of Pharmacy, Helwan University*

**Dr. Mohamed Soubhi Marzouk**

*Ass. Prof. of Natural Products Chemistry  
Department of Tannins Chemistry and Leather  
Technology, NRC*

**Dr. Magda Tohamy Ibrahim**

*Lecturer of Pharmacognosy  
Faculty of Pharmacy (Girls), Al-Azhar University*

**2005**

**PHARMACOGNOSTICAL AND BIOLOGICAL  
STUDIES OF *PLUMBAGO AURICULATA* Lam.**

**A Thesis**

**Submitted for the Degree of Doctor of  
Philosophy in Botany**

**By**

**Adawia Mahmoud Kitaz  
{M. Sc.}**

**Under Supervision**

**Dr. Magdy Mohamed Mourad**

*Ass. Prof. of Plant Taxonomy  
Faculty of Science, Ain -Shams University*

**Dr. Fatma Abdel Kader Moharram**

*Assi. Prof. of Pharmacognosy  
Faculty of Pharmacy, Helwan University*

**Dr. Mohamed Soubhi Marzouk**

*Ass. Prof. of Natural Products Chemistry  
Department of Tannins Chemistry and Leather  
Technology, NRC*

**Dr. Magda Tohamy Ibrahim**

*Lecturer of Pharmacognosy  
Faculty of Pharmacy (Girls), Al-Azhar University*

*Botany Department  
Faculty of Science, Ain-Shams University*

**2005**

## **Approval Sheet**

**Title of Thesis:** Pharmacognostical and biological studies of  
*Plumbago auriculata* Lam.

**Name of student:** Adawia Mahmoud Kitaz

**Degree:** Ph. Dr. in Science

**This thesis for the degree of Doctor of  
Philosophy in Botany**

**Has been approved by:**

**\*Dr. Magdy Mohamed Mourad**

**\*Dr. Fatma Abdel Kader Moharram**

**\*Dr. Mohamed Soubhi Marzouk**

**\*Dr. Magda Tohamy Ibrahim**

**(Committee in Charge)**

**Date:** / /2005

:

**A Thesis**  
**Submitted for the Degree of Doctor of**  
**Philosophy in Botany**

**Name:** adawia Mahmoud Kitaz

**Title:** Pharmacognostical and biological studies of *Plumbago auriculata* Lam.

**Degree:** Ph. Dr. in Science

**Under Supervision**

**Dr. Magdy Mohamed Mourad**  
*Ass. Prof. of Plant Taxonomy*  
*Faculty of Science, Ain-Shams University*

**Dr. Fatma Abdel Kader Moharram**  
*Ass. Prof. of Pharmacognosy*  
*Faculty of Pharmacy, Helwan University*

**Dr. Mohamed Soubhi Marzouk**  
*Ass. Prof. of Natural Products Chemistry*  
*Department of Tannins Chemistry and Leather*  
*Technology, NRC*

**Dr. Magda Tohamy Ibrahim**  
*Lecturer of Pharmacognosy*  
*Faculty of Pharmacy (Girls), Al-Azhar University*

**Head of Botany Department**  
**Prof Dr. Raefa A. Hasanin**

# Declaration

**This thesis has not been previously submitted for a degree  
at this or at any other University**

**Adawia Mahmoud Kitaz**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ  
رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ  
رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا

صَدَقَ اللَّهُ الْعَظِيمُ



***DEDICATION***

***TO SPIRIT OF MY FATHER***

***TO MY MOTHER***

***TO MY HUSBAND***

***TO MY DAUGHTERS***

***EKRAM, AREEJ AND***

***LUJAIN***

***TO MY LOVE COUNTRY***

***SYRIA***

# Acknowledgement

Thanks "*Allah*" for your mercy, help and continuous support to me.

I am deeply thankful to Arab Republic of Egypt for support and giving me all facilities to complete this work.

I would like to express my deep gratitude to ***Dr. Magdy Mohamed Mourad***, *Ass. Prof. of Taxonomy, Department of Botany, Faculty of Science, Ain-Shams University*, for his valuable supervision and kind help during the work.

My sincere thanks and gratitude to ***Dr. Fatma Abdelkader Moharram***, *Ass. Prof. of Pharmacognosy, Pharmacognosy Department, Faculty of Pharmacy, Helwan University*, for suggesting the point, intellectual stimulation, encouragement and continuous help. I am very grateful to her for her assistance and constructive criticism and providing me the excellent facilities for completing this work.

I wish to express my thanks and deepest appreciation to ***Dr. Mohamed Soubhi Marzouk***, *Ass. Prof. of Natural Products Chemistry, Department of Tannins Chemistry and Leather Technology, NRC*, for suggesting the point, his continuous supervision, fruitful assistance and constructive

criticism. I am very grateful to him for all valuable things I learned from him throughout this thesis.

My deepest thanks to **Dr. Magda Tohamy Ibrahim**, *Lecturer of Pharmacognosy, Faculty of Pharmacy (Girls)*, for her supervision, valuable help, continuous encouragement and constructive criticism which help me throughout this work.

Many thanks are also due to **Dr. Madiha Rabie Mahmoud** *Ass. Prof. of Pharmacology, Theodor Bilharz Research Institute*, for her assistance and guiding the biological effect.

Special thanks to **Prof. Dr. Galal H. El-Gemie**, *Prof. of Organic Chemistry and Vice Dean of Higher Studies and Research, Faculty of Science, Helwan University* and **Dr. Wafaa Abdou**, *Ass. Prof. of Organic Chemistry, Faculty of Pharmacy, Helwan University*, for providing laboratory facilities and their valuable help.

I am also greatly indebted to **Prof. Dr. Raeefa A. Hasanin**, *Head of Botany Department, Faculty of Science, Ain-Shams University*, for her encouragement throughout this work.

I would like to express my thanks to all **Members of the Department of Botany, Faculty of Science, Ain-Shams University**, for their valuable help and assistance.

Deepest gratitude to **My Family** for their help and continuous encouragement during the preparation of this work.

# Contents

|   | <b>Page</b> |
|---|-------------|
| <b>1. Introduction.....</b>                             | 1           |
| <b>2. Literature review.....</b>                        | 3           |
| 2.1. Phenolic compounds.....                            | 4           |
| 2.2. Anthraquinones and naphthoquinones .....           | 11          |
| 2.3. Triterpenes and sterols.....                       | 19          |
| 2.4. Coumarins.....                                     | 24          |
| 2.5. Miscellaneous constituents.....                    | 25          |
| 2.5.1. Carbohydrates.....                               | 25          |
| 2.5.2. Amino acids.....                                 | 25          |
| 2.5.3. Fatty acids .....                                | 25          |
| 2.6. Biological activities.....                         | 26          |
| <b>3. Taxonomy.....</b>                                 | 29          |
| <b>4. Materials, Methods and Apparatus.....</b>         | 33          |
| 4.1. Materials.....                                     | 33          |
| 4.1.1. Plant material.....                              | 33          |
| 4.1.2. Materials for biological study.....              | 33          |
| 4.1.2.1 Animals for biological study.....               | 33          |
| 4.1.2.2. Chemicals and kits for biological studies..... | 35          |
| 4.1.3. Authentic reference materials.....               | 37          |
| 4.1.3.1. Hydrocarbons.....                              | 37          |
| 4.1.3.2. Sterols and triterpenoidal compounds.....      | 38          |
| 4.1.3.3. Fatty acids.....                               | 38          |
| 4.1.3.4. Sugars and uronic acids.....                   | 38          |
| 4.1.3.5. Flavonoids and phenolic acids.....             | 38          |

|  | <b>Page</b> |
|--|-------------|
| 4.1.4. Materials for chromatography.....                                   | 38          |
| 4.1.5. Solvent systems.....  | 39          |
| 4.1.6. Spray reagents for PC and TLC.....                                  | 39          |
| 4.1.6.1. Ferric chloride reagent for phenolic compounds.....               | 39          |
| 4.1.6.2. Aluminium chloride reagent for flavonoids.....                    | 40          |
| 4.1.6.3. Diphenyl borinic acid ethanolamine complex.....                   | 40          |
| 4.1.6.4. Aniline hydrogen phthalate for sugars.....                        | 40          |
| 4.1.6.5. Sulphuric acid reagent for sterols and<br>triterpenes.....        | 40          |
| 4.1.7. Reagents for UV spectroscopic analysis of<br>flavonoids.....        | 41          |
| 4.2. Methods.....  | 41          |
| 4.2.1. Chromatographic methods.....  | 41          |
| 4.2.1.1. Paper chromatography .....  | 41          |
| 4.2.1.2. Thin-layer chromatography.....                                    | 42          |
| 4.2.1.3. Column chromatography .....                                       | 42          |
| 4.2.2. Acid hydrolysis of glycosides.....                                  | 43          |
| 4.2.3. Methylation of fatty acids.....                                     | 43          |
| 4.2.4. GLC of unsaponifiable matter.....                                   | 44          |
| 4.2.5. GLC of fatty acid methyl esters.....                                | 44          |
| 4.2.6. Preparation of 80% methanol extracts for<br>biological studies..... | 44          |
| 4.3. Apparatus.....  | 45          |

|  | <b>Page</b> |
|--|-------------|
| <b>5. Macro-and micromorphological studies of <i>Plumbago</i></b>                    |             |
| <i>auriculata</i> Lam. var. <i>alba</i> .....  | 47          |
| 5.1. Macromorphology.....  | 47          |
| 5.1.1. The root.....   | 47          |
| 5.1.2. The stem.....   | 47          |
| 5.1.3. The leaf.....   | 47          |
| 5.1.4. The inflorescence.....  | 50          |
| 5.1.5. The bract and bracteole.....  | 50          |
| 5.1.6. The flower.....   | 50          |
| 5.2. Micromorphology.....  | 54          |
| 5.2.1. The root.....   | 54          |
| 5.2.2. The stem.....   | 61          |
| 5.2.3. The leaf.....   | 68          |
| 5.2.4. The inflorescence.....  | 79          |
| 5.2.4.1 The bract and bracteole.....   | 79          |
| 5.2.4.2. The flower.....   | 82          |
| <b>6. Phytochemical screening.....</b>   | <b>99</b>   |
| <b>7. Investigation of lipoidal matter.....</b>                                      | <b>101</b>  |
| 7.1. Extraction of lipoidal matter.....  | 101         |
| 7.2. Preparation of unsaponifiable matter (USM) and fatty<br>acid methyl esters..... | 101         |
| 7.3. GLC of the unsaponifiable matter.....   | 103         |
| 7.4. GLC of fatty acid methyl esters.....  | 105         |
| <b>8. Isolation and identification of polyphenols from <i>P.</i></b>                 |             |
| <i>auriculata</i> var. <i>alba</i> .....   | 107         |

|   | <b>Page</b> |
|---|-------------|
| 8.1. Extraction and isolation of polyphenols from <i>P. auriculata</i> var. <i>alba</i> aerial parts..... | 107         |
| 8.2. Separation and structural elucidation of polyphenols.....  | 109         |
| <b>9. Biological activities.....</b>  | <b>169</b>  |
| 9.1. Preparation of extracts.....   | 169         |
| 9.2. Determination of median lethal dose (LD <sub>50</sub> ).....   | 169         |
| 9.3. Experimental design.....   | 169         |
| 9.3.1. Antischistosomal activity.....   | 170         |
| 9.3.2. Liver functions.....   | 179         |
| 9.3.3. Hepatoprotective activity.....   | 195         |
| 9.4. Evaluation of the anti-inflammatory activity.....  | 201         |
| 9.5. Antidiabetic activity .....  | 206         |
| <b>10. References.....</b>  | <b>209</b>  |
| <b>11. Summary.....</b>   | <b>217</b>  |
| <b>Arabic summary.....</b>  |             |

# Abbreviations

|                           |   |
|---------------------------|---|
| <b>Amm.</b>               | <b>Ammonia</b>  |
| <b><sup>13</sup>C-NMR</b> | <b><sup>13</sup>Carbon-Nuclear magnetic resonance</b>                           |
| <b><sup>1</sup>H-NMR</b>  | <b>Proton nuclear magnetic resonance</b>  |
| <b>2D/PC</b>              | <b>Two dimensional paper chromatography</b>                                     |
| <b>BAW</b>                | <b>Butanol: Acetic acid: Water</b>  |
| <b>BIW</b>                | <b>Butanol: Isopropanol: Water</b>  |
| <b>CC</b>                 | <b>Column chromatography</b>  |
| <b>ESI</b>                | <b>Electrospray ionization</b>  |
| <b>GLC</b>                | <b>Gas liquid chromatography</b>  |
| <b>LD<sub>50</sub></b>    | <b>Lethal dose &amp; the dose causing death of<br/>50% of the tested animal</b> |
| <b>NA/PE</b>              | <b>Natural product/ polyethylene glycol</b>                                     |
| <b>OD</b>                 | <b>Optical density</b>  |
| <b>PC</b>                 | <b>Paper chromatography</b>   |
| <b>PPC</b>                | <b>Preparative paper chromatography</b>   |
| <b>Rpm</b>                | <b>Revolution per minute</b>  |
| <b>TLC</b>                | <b>Thin layer chromatography</b>  |

## List of Tables

| <b>Tables</b>   | <b>Page</b> |
|---|-------------|
| 1. Phenolic compounds reported in <i>Plumbago</i> species.....                                      | 4           |
| 2. Anthraquinones and naphthoquinones reported in <i>Plumbago</i> species.....                      | 11          |
| 3. Triterpenes and sterols reported in <i>Plumbago</i> species.....                                 | 19          |
| 4. Conformation and configuration of the common glycoside moieties.....                             | 23          |
| 5. Biological effects of the constituents and extracts of <i>Plumbago</i> species .....             | 26          |
| 6. Phytochemical screening of aerial parts of <i>P. auriculata</i> and its var. <i>alba</i> .....   | 100         |
| 7. Percentage of USM and TFA in aerial parts of <i>P. auriculata</i> and its var. <i>alba</i> ..... | 102         |
| 8. GLC of hydrocarbons and sterols in USM of <i>P. auriculata</i> and its var. <i>alb</i> .....     | 104         |
| 9. GLC analysis of fatty acid methyl esters of <i>P. auriculata</i> and its var. <i>alba</i> .....  | 106         |
| 10. Chromatographic properties and UV spectral data of P <sub>5</sub> and P <sub>6</sub> .....      | 140         |