



Chemical and Biological Investigation of Certain Species Belonging to Genus *Cassia* Family (Caesalpiniaceae)

A Thesis Submitted

In Partial Fulfilment of the Requirements
for the Degree of Master in Pharmaceutical Sciences

(In Pharmacognosy)

By

Safaa Abdel Raouf Hafez Ali

Bachelor of Pharmaceutical Sciences,
Faculty of Pharmacy, October 6 University, 2005

Under the Supervision of

Dr. Ahmed Attia Seida Ph. D.

Professor of Pharmacognosy
Faculty of Pharmacy
Cairo University
President of October 6 University

Dr. Nahla A. Ayoub Ph. D.

Professor of Pharmacognosy
Faculty of Pharmacy
Ain Shams University

Dr. Samir Osman

Assoc. Professor of Pharmacognosy
Faculty of Pharmacy
October 6 University

Department of Pharmacognosy
Faculty of Pharmacy
Ain Shams University
Abbasia, Cairo, Egypt
2019

To the memory of my dear father

Abdel Raouf Hafez Ali

And to my beloved mother

ACKNOWLEDGEMENT

I am deeply grateful to God, by the grace of whom this work was possible and whose guidance and protection secured every step of the road.

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:

*I would like to dedicate my appreciation, sincere gratitude and great indebtedness to **Prof. Dr. Ahmed Attia Seida**, President of October 6 University and Professor of Pharmacognosy, Faculty of Pharmacy Cairo University, for supervising the work, and for his kind effort and help and indispensable advice and guidance.*

*I wish to express my deepest thanks, love and appreciation to **Prof. Dr Nahla A. Ayoub**, Professor of Pharmacognosy, Ain Shams University for suggesting the research topic, continuous encouragement during the course of this work and for the systematic guidance and great effort she did during the work. I am indebted to her with every single word in this thesis as I feel lucky to be her student.*

*I would like to express my sincere gratitude to **Dr. Samir Osman**, Ass. Prof. of Pharmacognosy, Faculty of Pharmacy, October 6 University for his generous advice and encouragement throughout the work.*

*My deepest thanks and sincere appreciation, gratefulness and indebtedness to my God father **Prof. Dr. Abdel Naser Singab**, vice president of Ain Shams University, for his great support, advice and guidance and whose support was essential to complete this work.*

*I present my deepest thanks and gratitude to my other God father, **Prof. Dr. Mahmoud Koheil** professor of pharmacognosy, Dean of faculty of pharmacy, October 6 university, for his co-operation , kind support , constant encouragement and valuable time .*

*My sincere gratitude to my dear doctors in the pharmacognosy department, October 6 university **Dr. Alaa Elhadad, Dr. Jilan Nazim, Dr. Ayat Emad, Dr. Amr Saad Eldin, Dr. Lina Jamil, Dr. Shereen Sameh, Dr. Khaled Abdel haleem** for their valuable advice, continuous support and kind assistance.*

*Deep gratitude and thanks to **Prof. Dr. Farghaly Omar**, professor and head of pharmaceutical chemistry department ,October 6 university , for his kind support and assistance in the pharmaceutical chemistry investigations.*

*I would like to thank my dear colleagues and friends; **Dr. Eman Eldeeb, Dr. Ahmed Gaweesh, Dr. Esraa Zakaria, Dr. Albraa Akram, Dr. Nourhan Hassan** for their continuous moral support, encouragement and love.*

*An acknowledgement to **Ass. Prof. Mohamed El raey, Dr. Tamer Rgab and Dr. Mahmoud Emam**, the national research centre, for their great help and effort.*

*A special word of gratitude to the great staff members of pharmacognosy department, Ain Shams University, dear **Prof. Dr. Omaima El Dahshan, Prof. Dr. Sherwiet, Ass. Prof. Dr. Rola** and **Ass. Prof. Dr. Mohamed Ashour** whose kindness and help were very valuable for me.*

*A special thank you note to my dear love and sisters **Dr. Iriny Ayoub, Dr. Naglaa Saad Eldin, Nada Mohammed** and my dear brother **Dr. Ahmed Essam** lecturers of pharmacognosy for their unforgettable support, generous advice and patience.*

*My deepest thanks and gratitude goes to dear my sister **Dr. Noha Swilam** and **Dr. Khaled Neamatalla**, Lecturers of pharmacognosy, British University in Egypt (BUE), for their indispensable help and great efforts in data interpretation.*

I wish also to thank **Dr. Haitham Ali** for his kind help and support.

Appreciation to **Ass. Prof. Dr. Kareem Eltobgy** and **Mr. Magdi** for their valuable help and efforts in the analysis work.

Deep gratitude and thanks to **Prof. Dr. Mohamed Farag**, Professor of Pharmacognosy, Cairo University and The American University in Cairo (AUC) for his kind support, valuable time, patience and help. I am honored to have had the chance to work with him and learn a lot of knowledge, science and morals through dealing with such a great professor of pharmacognosy and a great teacher of life.

I also express my thanks to the technicians of the pharmacognosy department, October 6 University; **Mrs. Amany Esa**, **Mr. Mohamed Fathy**, **Mr. Alaa Madbouly**, **Samah**, **Om Mahmoud**, **Om Nasser**, **Om Tamer**, **Kamel**. for their kind help and support.

A word of thanks and appreciation to my soul mate and friend **Dr. Sara Mohamed Hashim** for her moral support, endurance and lots of help.

I also wish to thank my second mother, sister and beloved friend **Dr. Sherifa Fahmy** Ass. Prof of Pharmacognosy, Cairo University and October 6 University for her moral support in completing this work and for her love and care.

Finally, I am deeply thankful to my dear family specially my beloved **mother**, whom her unconditional love and support during the hardest times gave me the light to see my way and to the memory of my late father whose soul was the inspiration for my soul to go on and never stop. Thank you **mum** and **dad**.

Safaa

Table of Contents

Item	Page
LIST OF ABBREVIATIONS.....	I
LIST OF FIGURES.....	III
LIST OF TABLES.....	VII
INTRODUCTION	1
REVIEW OF LITERATURE ON GENUS CASSIA	
a- Chemical review of literature	5
b- Biological review of literature	36
TAXONOMY OF GENUS CASSIA.....	40
MATERIAL, APPARATUS AND METHODS.....	46
CHAPTER (1): PHYTOCHEMICAL STUDY OF	
CASSIA GLAUCA LEAVES	74
Part I: UPLC-MS of chemical profile of	
<i>Cassia glauca</i> leaves.....	74
Part II: Phytochemical screening of <i>Cassia</i>	
<i>glauca</i> leaves.....	90
Part III: Value added components from plant	
marc of <i>Cassia glauca</i> leaves	107
CHAPTER (2): GREEN SYNTHESIS OF	
SILVER NANO PARTICLES	130
CHAPTER (3): BIOLOGICAL STUDY OF CASSIA GLAUCA	138
GENERAL SUMMARY	163
CONCLUSION AND RECOMMENDATIONS	167
REFERENCES	169
ARABIC SUMMARY	\

List of Abbreviations

AcOH-6%	6% acetic acid
ANOVA	Analysis of variance
Ag NPs	Silver nanoparticles
AR	Aldose Reductase
AO	Acridine orange
ASP	Aspartate
¹³C-NMR	Carbon-13 Nuclear Magnetic Resonance
CC	Column chromatography
CrI	Crystallinity index
CID	Collision-induced dissociation
<i>d</i>	Doublet
<i>dd</i>	Doublet of doublet
DMF	Dimethylformamide
DMSO-<i>d</i>₆	Deuterated Dimethylsulfoxide- <i>d</i> ₆
DOX	Doxorubicin
<i>eq.</i>	Equatorial
ESI-MS	Electro-Spray Ionization Mass Spectrometry
ESI	Electrospray ionization
ELISA	Enzyme linked immunosorbent assay
EB	Ethidium bromide
FTIR	Fourier-transform infrared spectroscopy
FTMS	Fourier transform MS
FBS	Fetal bovine serum
GLU	Glutamine
HepG2	Hepatocellular carcinoma cell line
¹H-NMR	Proton Nuclear Magnetic Resonance
HPLC	High-performance liquid chromatography
HESI	Heated electrospray ion source
HR	High resolution
IC₅₀	Inhibitory concentration by 50 %.
<i>J</i> value	Coupling constant
LYS	Lysine
LEU	Leucine

List of Abbreviations

LIT	Linear ion trap
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
MCF-7	Human breast adenocarcinoma cell line
MPa	Megapascal
MF	Molecular formula
MSn	Tandem mass spectra
NMR	Nuclear Magnetic Resonance Spectrometer
NCC	Nano crystals cellulose
PC	Paper Chromatography
PDB	Protein data bank
PDA	Photodiode-array
PHE	phenylalanine
<i>q</i>	quartet
R_f	Retardation factor
R_t	Retention time
RP	Reversed phase
<i>s</i>	singlet
SEM	Scanning electron microscopy
SPR	Surface plasmon resonance
SER	Serine
<i>t</i>	triplet
TLC	Thin Layer Chromatography
TEM	Transmission Electron Microscope
UV	Ultraviolet
<i>UHPLC-MS</i>	Ultra-high performance liquid chromatograph tandem mass spectrometry
XRD	X-ray diffraction
2D-PC	Two dimensional paper chromatography

LIST OF FIGURES

Figure	Page No.
1. Chemical structure of reported anthraquinones, anthracenes, their compounds have been derivatives isolated from different <i>Cassia</i> species	10
2. Chemical structure of reported phenolic compounds have been isolated from different <i>Cassia</i> species	24
3. Chemical structure of reported miscellaneous compounds have been isolated from different <i>Cassia</i> species	31
4. a) Tree of <i>Cassia glauca</i> x= 0.026, b) Leaves of <i>Cassia glauca</i> x= 0.9, c) Compound leaf of <i>Cassia glauca</i> x= 0.44, d) Leaflet of <i>Cassia glauca</i> x= 1, e- Flower of <i>Cassia glauca</i> , f- Pods of <i>Cassia glauca</i>	45
5. Procedure of Separation of macromolecules of the biomass of <i>Cassia glauca</i> leaves marc.....	64
6. UPLC-MS/MS chromatogram of the methanol extract of <i>Cassia glauca</i> leaves	75
7. MS/MS spectrum of Caffeoyl hexoside at [M-H]-341	77
8. MS/MS spectrum of Quercetin rhamnosyl-rutinoside at [M-H]- m/z 755	78
9. MS/MS spectrum of Kaempferol rhamnosyl-rutinoside at [M-H]- m/z 739	79
10. Expected fragmentation pattern of Kaempferol rhamnosyl-rutinoside.....	80
11. MS/MS spectrum of Quercetin rutinoside (Rutin) at [M-H]- m/z 609	81
12. Expected fragmentation pattern of Quercetin rutinoside (Rutin).....	82

13. MS/MS spectrum of Quercetin hexoside of [M-H]- at m/z 463.....	83
14. Expected fragmentation pattern of Quercetin hexoside.....	84
15. MS/MS spectrum of Kaempferol rutinoside at [M-H]- m/z 593	85
16. MS/MS spectrum of Kaempferol hexoside at [M-H]- m/z 447	86
17. MS/MS spectrum of at Isorhamnetin [M-H]- m/z 315	87
18. MS/MS spectrum of Eriodictyol at [M-H]- m/z 287	88
19. MS/MS spectrum of Apigenin at [M-H]- m/z 269	89
20. FTIR of <i>Cassia glauca</i> extract	91
21. Schematic figure for separation and isolation of compound 1 and 2	92
22. ¹ H NMR spectrum of compound 1	96
23. Apt- NMR spectrum of compound 1	97
24. Positive ESI-MS spectrum of Compound 1	98
25. Negative ESI-MS spectrum of Compound 1	98
26. Chemical structure of isolated kaempferol 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (kaempferol 3-O- β -D-rutinoside) from <i>Cassia glauca</i> leaves	99
27. ¹ H NMR spectrum of compound 2	102
28. ¹ H NMR spectrum of compound 2	103
29. Apt- NMR spectrum of compound 2	104
30. Positive ESI-MS spectrum of Compound 2	105
31. Negative ESI-MS spectrum of Compound 2	105

32. Quercetin 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (Rutin) isolated from <i>Cassia glauca</i> leaves	106
33. Structure of cellulose	107
34. Structure of hemicellulose	110
35. Structure of lignin	111
36. Yield of extracted crude cellulose, hemicellulose and lignin from marc residue (w/w%) of <i>Cassia glauca</i> leaves	112
37. FTIR of cellulose, hemicellulose and lignin	115
38. FTIR spectroscopy of (a-Bleached b- α c- Nano crystalline) cellulose.....	118
39. X-ray diffraction of (a-Bleached, b- α and c-Nano crystalline) cellulose.....	120
40. Scanning electron microscope of a) bleaching cellulose, b) α -cellulose and c) nano crystalline cellulose.....	122
41. Ag NPS synthesized using total aqueous extract, methanol fraction and remaining aqueous fraction of <i>Cassia glauca</i> leaves	132
42. Synthesis of Ag NPs for the fractions of <i>C. glauca</i>	133
43. The SPR band of Ag nanoparticles using total aqueous extract, methanol fraction and remaining aqueous fraction recorded by UV-vis spectra as a function of concentrations of <i>Cassia glauca</i> leaves	135
44. The TEM images of total extract, methanol fraction and remaining aqueous fraction) of <i>Cassia glauca</i> leaves	136
45. Dose response curves of cytotoxic study before and after nano (total extract, methanol fraction, remaining aqueous fraction), kaempferol rutinoside and rutin on HepG2	140

46. Dose response curves of cytotoxic study before and after nano (total extract, methanol fraction, remaining aqueous fraction), kaempferol rutinoside and rutin on MCF7	141
47. Dose response curves of cytotoxic study of kaempferol rutinoside and rutin on Caco	142
48. Surface map of SL0101 (green) co-crystallized with RS6K 2 (3aub) ; and the corresponding interaction diagram	146
49. Surface map of Kampferol-3-O-B-rutenoside (yellow) and the co-crystallized SL0101with RS6K 2 (3aub) ; and the corresponding interaction diagram	148
50. Surface map of Rutin (yellow) and the co-crystallized SL0101with RS6K 2 (3aub) ; and the corresponding interaction diagram	148
51. Mode of Cell death of Rutin, Kaempferol rutinoside and/or DOX on cell death mode and their effects on the percentage of different cell populations	150
52. MCF-7 cells stained by Acridine orange/ ethidium bromide	151
53. Cell Viability assay of combining kaempferol rutinoside with DOX	152
54. Role of Aldose reductase in the polyol pathway	153
55. 2D and 3D interactions of a-Synthetic Aldose reductase inhibitors and b- Natural flavone Aldose reductase inhibitors	160

List of Tables

Table	Page No.
1. Reported Anthraquinones, anthracenes and their derivatives isolated in different <i>Cassia</i> species	5
2. Phenolic compounds reported in different <i>Cassia</i> species	20
3. Some miscellaneous compounds reported in different <i>Cassia</i> species	28
4. Biological activities of genus <i>Cassia</i>	36
5. UPLC-MS/MS profile of the leaf extract of <i>Cassia glauca</i>	76
6. Phytochemical screening of <i>Cassia glauca</i> leaves	90
7. Composition of different prepared tablet (total weight=100mg)	124
8. Quality control evaluation for different prepared tablets	126
9. Release of aspirin from different prepared tablets formulae using lactose and cellulose derivatives (Avicel & NCC) as a binder	127
10. The results of the Cytotoxic activity of the different extracts, fractions and isolated compounds before and after nano formulation on HepG-2, MCF-7 and Caco cell lines as determined by MTT assay	142
11. Docking Scores & interaction modes of Camphor and Carvotanacetone	147
12. The % of viable, apoptotic and necrotic population cells	149
13. Microbiological screening for the antimicrobial activity of the different extracts, fractions and isolated compounds before and after nano formulation are against <i>Staphylococcus aureus</i> , <i>E.coli</i> , <i>Pseudomonas aeruginosa</i> and <i>Candida albicans</i> by agar diffusion method	161

Introduction

Since ancient times, several societies have resorted to nature, mainly to plants as medical and health sources. Today, a great percentage of the world population, in particular in developing countries, uses plants for facing primary needs of medical assistance (Tene *et al.*, 2007).

Family Leguminosae is one of the largest families of the flowering plants. It comprises about 650 genera and 18000 species (Hu *et al.*, 2000). The family is divided into three sub-families: Caesalpinioideae, Papilionoideae and Mimosoideae. These sub-families are now treated as independent families due to their large size and named, Caesalpiniaceae, Papilionaceae, and Mimosaceae (Gledhill, 2008). The name Caesalpinioideae is derived from the generic name *Caesalpinia*. The Caesalpinioideae are mainly trees distributed in the moist tropical areas.

Caesalpinaceae represents approximately 11% of the known legume flora, with 152 genera and 2800 species (Willis, 1973).

The members of this family are characterized by the legume type of fruits (pods), in which the seeds are housed. They are distributed throughout the world in almost all habitats; however the greatest diversity of the legumes is in the tropical and subtropical areas (Ali *et al.*, 2001).

Phenolic compounds are commonly found in both edible and non-edible plants of family Leguminosae and they have been reported to have multiple biological effects. The flavonoids and other phenolics