PHYTOCHEMICAL AND BIOLOGICAL STUDY ON CERTAIN PLANTS BELONGING TO FAMILY SCROPHULARIACEAE

Thesis Submitted to

Faculty of Pharmacy
Ain Shams University

In partial fulfillment of the requirements
For the degree of
Doctor of Philosophy in Pharmaceutical Sciences
(In Pharmacognosy)

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ACKNOWLEDGEMENT

I would like to express my gratitude and appreciation to the members of the advisory committee;

Prof. Dr. Abdel Nasser B. Singab, *Professor of Pharmacognosy, Dean of Faculty of Pharmacy, Ain Shams University for suggesting the research point. Words are not enough to express my deep gratitude and profound thanks to him for his kind supervision, indispensable advice, valuable comments, generous support, sincere guidance and continuous encouragement throughout the course of study, setting an example to what a dedicated professor, scientist and advisor should be. I am truly proud to be one of his students. His constructive criticism and suggestions helped me to improve this work through the experimental investigations as well as writing and revising the thesis.*

Dr. Mohamed Lotfy Ashour *Lecturer of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, for his kind supervision, indispensable advice, valuable comments. No words can express my gratitude to him for his helpful suggestions, valuable assistance and constant guidance throughout the whole study. His friendly attitude and meticulous observations have made the work very interesting.*

Special thanks to ***Dr. Rola Milad Labib****, Lecturer of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, for her great effort, endless patience, helpful guidance, constant support and continuous encouragement while working and accomplishing the botanical section.*

Great thanks to ***Prof. Dr. Hesham El-Beshbishy****, Professor of Biochemistry, Faculty of Applied Medical Sciences, Taibah University, Al-Madinah Al-Munwarah, Saudi Arabia, for hosting the in vivo biological study.*

Many thanks to ***Dr. Mohamed El-Shazley****, Lecturer of Pharmacognosy, Faculty of Pharmacy, Ain Shams University for carrying out the in vitro cytotoxicity and anti-allergic assays in College of Pharmacy, Kaohsiung Medical University, Taiwan.*

Alot of thanks to ***Dr. Sherif Ebada,*** *Lecturer of Pharmacognosy, Faculty of Pharmacy, Ain Shams University for carrying out the LC-MS runs at Institute for Pharmaceutical Biology and Biotechnology, Heinrich-Heine University of Duesseldorf, Germany.*

Also, I would like to express my deep thankfulness to both ***Prof. Dr. Michael Wink,*** *Professor of Biology, Head of Biology Department and Director at the Institute of Pharmacy and Molecular Biotechnology, Heidelberg University; for hosting the GLC-MS, GLC-FID and NMR analysis and* ***Prof. Dr. Fang-Rong Chang****, Professor of Pharmacognosy and Director of Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, for hosting the cytotoxicity and anti-allergic assays at their institutes.*

Special thanks to all my dear ***professors*** *especially* ***Prof. Dr. Mohamed M. Al-Azizi****, Professor of Chemistry of Natural Drugs, Faculty of Pharmacy, Ain Shams University for his brilliant suggestions, sincere guidance and endless valuable assistance to us all in the department, we all considered him as our great teacher and professor in all aspects of life and above all our dear loving father.*

Many thanks go to my ***colleagues*** *for their cooperation, support, valuable comments and the friendship we share, hoping to continue so ever.*

I cannot find enough words to express my deep love and thanks for ***my parents****: for their continuous care, love and support. They sacrificed their time and efforts to help me to achieve this work.*

Finally, I would like to thank my dear husband ***Dr. Albert Victor****, for his continuous encouragement, support and most of his patience for which I am truly grateful.*

I dedicate this work to my beloved children ***Fady*** *and* ***Marlie,*** *the most precious treasure in my life****,*** *whom I give my love, care and I hope that they will always be proud of me.*

Fadia Salah Youssef

2014

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List of abbreviations

ADP	Adenosine diphosphate
ALT	Alanine transaminase
ANOVA	One-way analysis of variance
APT	Attached proton test
AST	Aspartate transaminase
ATCC	American type culture collection
BSA	Bovine serum albumin,
cAMP	Cyclic adenosine monophosphate
CCl_4	Carbon tetrachloride
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
CFU	Colony forming unit
^{13}C-NMR	Carbon-13-Nuclear Magnetic Resonance
COPD	Chronic obstructive pulmonary disease
COX-1	Cycloxygenase-1
COX-2	Cycloxygenase-2
CPE	Cytopathic effect
CPR	Coronary perfusion rate
CTAB	N-cetyl-N, N, N-trimethylammonium bromide
d	Doublet
dd	Doublet of doublet
DMEM	Dulbecco's Modified Eagle Medium
DMSO-d_6	Dimethylsulfoxide-d_6
DNA	Deoxyribonucleic acid
2D-NMR	Two-dimensional nuclear magnetic resonance spectroscopy
DNP	Dinitrophenyl
DNP-BSA	Dinitrophenyl -Bovine Serum Albumin
dNTP'S	Deoxyribonucleotide triphosphates
$DPPH^{\cdot}$	2,2-Diphenyl-1-picrylhydrazyl radical
EDTA	Ethylenediaminetetraacetic acid
EI-MS	Electron ionization or Electron impact- Mass Spectrometry
ELISA	Enzyme-linked immunosorbent assay
EMF	*Eremophila maculata* flower essential oil
EML	*Eremophila maculata* leaves essential oil
EMM	*Eremophila maculata* leaves total methanol extract
EMSD	*Eremophila maculata* dry stem essential oil
EMSF	*Eremophila maculata* fresh stem essential oil
ESI-MS	Electrospray ionization – Mass Spectrometry
eV	Electron volt
FBG	Fasting blood glucose
Fig.	Figure
g	Gram
GC	Gas liquid chromatography
GC-FID	Gas liquid chromatography– flame ionization detector
GC-MS	Gas liquid chromatography–mass spectrometry

GLB	Glibenclamide
Glc.	Glucose
GLUT2	Glucose transporter isoform 2
GPx	Glutathione peroxidase
GS	Genetic similarity
GSH	Reduced Glutathione
HCMV	Human cytomegalovirus
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
H,H-COSY	H,H Correlated spectroscopy
HMBC	Heteronuclear multiple-bond correlation spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HPLC	High Performance Liquid chromatography
Hrs.	Hours
HSQC	Heteronuclear Single Quantum Coherence
HSV-1	Herpes simplex virus 1
5-HT	5-Hydroxytryptamine
Hz	Hertz
IC_{50}	The half maximal inhibitory concentration
IgE	Immunoglobulin E
i.p.	Intraperitoneally
IU/ml	International unit/ml
J value	Coupling constant
Kg	Kilogram
KI	Kovats index
l	Litre
LC-MS	Liquid chromatography–mass spectrometry
5-LO	5- Lipoxygenase.
LPO	Lipid peroxidation
m	Meter
m	multiplet
MBC	Minimum bactericidal concentration
mg	Milligram
mg/kg	Milligram per kilogram
mg/ml	Milligram per milliliter
MHz	Megahertz
MIC	Minimum inhibitory concentration
min.	Minute
ml	Milliliter
ml/min	Milliliter per minute
mm	Millimeter
mM	Millimole
MMC	Minimum microbicidal concentration
MRSA	Methicillin – resistant *Staphylococcus aureus*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
m/z	Mass to charge ratio
NAD+	Nicotinamide adenine dinucleotide

ηgm	Nanogram
nm	Nanometer
NT	Not tested
PBS	Phosphate buffered saline
PFU	Plaque-forming unit
p-NAG	*p*-Nitrophenyl-N-acetyl-D-glucosaminide
ppm	Part per million
PTLC	Preparative Thin layer chromatography
RAPD-PCR	Random Amplified Polymorphic DNA- Polymerase Chain Reaction
RCMB	Regional center of mycology and biotechnology
R_f	Retardation factor
RI	Retention index
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RP-18	Reversed phase-18
rpm	Revolution per minute
RPMI	Roswell Park Memorial Institute
RRV	Ross River Virus
s	Singlet
S.D.	Standard deviation
SOD	Superoxide dismutase
SM	Secondary metabolites
SRB	Sulforhodamine B
STE	Standard error of the mean
STZ	Streptozotocin
t	Triplet
TAC	Total antioxidant capacity
TAM	Tamoxifen Citrate
TBARS	Thiobarbituric acid reactive substances
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TNF-α	Tumor necrosis factor- alpha
T.S.	Transverse section
µg	Microgram
µg/ml	Microgram/milliliter
µl	Microliter
µm	Micrometer
µM	Micromole
UV	Ultraviolet
VRE	Vancomycin- resistant *Enterococcus*
VSV	Vesicular Stomatitis virus
v/v	Volume per volume
v/w	Volume per weight
XO	Xanthine oxidase
δ	Chemical Shift

Introduction

Natural products are considered the major source of lead compounds required for future drug development programs (Newman et al., 2003). Throughout ages, humans have traditionally relied on plants, animals and minerals for their basic needs, such as for food, protection against enemies, hunting, healing of infections and health disorders. A number of traditional medicinal systems that have been used for centuries have evolved and today are a source of interesting drugs for Phytotherapy (Singab et al., 2013).

Plant materials remain an important component in combating serious diseases in the world; from therapeutic approaches to several pathologies. (Iwu et al., 1999). Nowadays, it is very interesting to emphasize that despite of the tremendous increase in manufacturing synthetic and/or semi-synthetic therapeutic agents to face the global demand for new drugs, approximately 80% of the world's inhabitants rely mainly on traditional herbal medicines for their health care (Owolabi et al., 2007).

Reports on pharmacological effects of medicinal plants are growing almost exponentially. However, it is very difficult to attribute the pharmacological activity in a multi-component mixture, as in plant extracts consisting of a diversity of secondary metabolites, to only a single compound of the extract, but there is good evidence that the secondary metabolites, present in a mixture, exhibit additive or even synergistic effects. They are able to interfere with many molecular targets in the cells (Wink, 2008).

Scrophulariaceae is a large family with almost 87 genera and 4800 species. Plants of this family are widely used in traditional medicine and phytotherapy (Kadereit, 2004). The corresponding biological activities are related to their richness in secondary metabolites such as phenylpropanoids, iridoid glucosides, and terpenoids with anti-inflammatory, antinociceptive, wound healing and antimicrobial activities (Diaz et al., 2004, Akdemir et al., 2011). In addition, many species are rich in polyphenols, flavolignans and phenolic acids, which exhibit antiprotozoal (Tasdemir et al., 2005) antioxidant (Singab et al., 2005, Jeong et al., 2009), antibacterial (Liu et al., 2006) and cytotoxic properties (Afifi et al., 1993).

Eremophila (Scrophulariaceae) is an endemic Australian genus with 214 species, which is commonly known as Fuchsia bush, Emu bush or Poverty bush. Plants of this genus played an important role for the Australian Aborigines who used them widely for medicinal and ceremonial purposes (Singab et al., 2013).

Phytochemical investigations of the genus *Eremophila* lead to isolation and identification of more than 200 secondary metabolites (SM) from several classes (Ghisalberti, 1993). Major SM include diterpenes (eremane, cembrane, decipiane, and viscidane type); others are triterpenoids, verbascosides, cyanogenic glucosides, fatty acids, phenylpropanoids, lignans, and flavonoids (Ghisalberti, 1994) to which many biological and pharmacological activities such as antimicrobial (Tomlinson and Palombo, 2005), antiviral (Semple et al., 1998), antiproliferative (Beattie et al., 2011), anti-inflammatory (Liu et al., 2006), and immunomodulatory activities (Rogers et al., 2000) have been attributed.

Although, many studies have been carried out on many species of this genus and have generated immense data about the chemical composition and corresponding biological activity of extracts and isolated secondary metabolites, there are still a lot to be explored.

Reports on the botanical study of many *Eremophila* species were fragmentary. Lack of comprehensive botanical study on different plant organs makes it obligatory to carry out a comparative study in order to find out the main diagnostic features of the different organs of its species to help in their identification. Botanical characterization of closely related plant species is nowadays greatly supported *via* examination of various decisive genetic criteria such as isoenzymes, DNA or seed proteins (Rogl et al., 1996). The concept of DNA fingerprinting has been, in this respect, increasingly applied to establish the ancestry of plants. It is reported as a promising tool for the authentication of medicinal plant species and especially useful in species or varieties that are morphologically and/or phytochemically indistinguishable (Rola Milad et al., 2013).

Also, fewer studies have been done to identify the volatile oil composition of some *Eremophila* species such as *E. longifolia* (Smith et al., 2010) and *E. mitchellii* (Beattie et al., 2011). To the best of our knowledge, the essential oil composition as well as the antimicrobial activity of *E. maculata* flower, leaf and stem oils have not been reported yet.

Concerning the biological activities of many *Eremophila* species, there are a lot to be investigated, like determining the effect of these plants, their extracts as well as their isolated compounds on the blood glucose level, their antibacterial, antiviral, antifungal, ant-inflammatory and cytotoxic activities. Also, their effect on the liver has to be considered where liver is the chief site for intense metabolism and excretion.