Phytochemical and Biological Studies on Some Plants Belonging to Family Araliaceae

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)

By

Naglaa Saad Eldin Mostafa

B. Pharm. Sci.
Faculty of Pharmacy, Ain Shams University, 2007
M. Pharm. Sci
Faculty of Pharmacy, Ain Shams University, 2013

Department of Pharmacognosy Faculty of Pharmacy Ain Shams University Abbasia, Cairo, Egypt 2018

Under the Supervision of

Prof. Dr. Abd El-Nasser Badawy Singab

Vice President of Ain Shams University for Postgraduate Affairs

Chairman of Center for Drug Discovery, Research and Development

Professor of Pharmacognosy

Faculty of Pharmacy

Ain-Shams University

Dr. Sherweit Hamed El-Ahmady

Associate Professor of Pharmacognosy
Faculty of Pharmacy
Ain-Shams University

Dr. Mohamed Lotfy Ashour

Associate Professor of Pharmacognosy
Faculty of Pharmacy
Ain-Shams University

Dr. Haidy Abdel Moneim Gad

Associate Professor of Pharmacognosy
Faculty of Pharmacy
Ain-Shams University

Department of Pharmacognosy Faculty of Pharmacy Ain Shams University Abbasia, Cairo, Egypt 2018

Table of Contents

Content	Page
List of Tables	i
List of Figures	iii
List of Abbreviations.	vi
Introduction	1
Literature review	4
1. Traditional uses	5
2. Biological activities	5
3. Phytochemistry of the genus <i>Polyscias</i>	12
	42
Taxonomy	42
Material apparatus and methods	45
1. Material	45
2. Apparatus	48
3. Methods	50
Chapter 1: Chemical Composition of the Leaf Methanol Extract of <i>Polyscias guilfoylei</i>	60
(Araliaceae)	60
1. Experimental	
2. Results and discussion	63
2.1. Phytochemical screening of <i>Polyscias guilfoylei</i> leaves	63
2.2. Phytochemical study of <i>Polyscias guilfoylei</i> leaves	64
2.2.1. Compounds isolated from the <i>n</i> -hexane fraction of the <i>P. guilfoylei</i> leaves	
total methanol extract	66
2.2.1.1. Compound 1 (<i>Ent</i> -labda-8(17), 13-diene-15, 18-diol)	66
2.2.1.2. Compound 2 (Stigmasterol)	73
2.2.1.3. Compound <u>3</u> (Spinasterol)	77
2.2.1.4. Compound 4 (N-(1, 3-dihydroxyoctadecan-2-yl) palmitamide)	83
2.2.1.5. Compound <u>5</u> (Panaxydiol)	95
2.2.2. Compounds isolated from the methylene chloride fraction of the <i>P. guilfoylei</i>	- -
leaves total methanol extract	99

	2.2.2.1. Compound $\underline{6}$ (3- O - β -D-glucopyranosylstigmasta-5, 22-diene-3- β -ol)
	2.2.2.2. Compound 7 ((8Z)-2-(2 hydroxypentacosanoylamino) octadeca-8-ene-1,
	3, 4-triol)
2	2.2.3. Compounds isolated from the ethyl acetate fraction of the <i>P. guilfoylei</i> leaves
t	total methanol extract
	2.2.3.1. Compound 8 (4-hydroxybenzoic acid)
	2.2.3.2. Compound $\underline{9}$ (Tamaraxetin 3, 7-di- O - α -L-rhamnopyranoside)
	2.2.4. LC-ESI-MS analysis of <i>n</i> -hexane fraction of <i>P. guilfoylei</i> leaves
2	2.2.5. LC-ESI-MS analysis of methylene chloride fraction of <i>P. guilfoylei</i> leaves
2	2.2.6. LC-ESI -MS analysis of butanol fraction of <i>P. guilfoylei</i> leaves
•	
_	er 2: Biological Investigation of the leaf Methanol Extract, Fractions and d Compounds of <i>Polyscias guilfoylei</i> (Araliaceae)
isolate	a Compounds of 1 olystus guilloyiet (Aranaceae)
1.	Experimental
2.	Results
	2.1. Anti-microbial activity
	2.2. Cytotoxicity
	2.3. <i>In-vitro</i> histamine release inhibitory activity
	2.4. In viiro and aging activity
3.	Discussion
-	er 3: Chemical Composition and Biological activity of the Leaves of <i>Polyscias</i>
guilfoy	lei and Polyscias balfouriana Essential oil
1. Resu	ılts
	ussion
	al Summary
	isions and recommendations
	nces
	e summary

List of Tables

No	Table	Page
1.	Summary of the most relevant reported pharmacological activities of <i>Polyscias</i>	
	species	9
2.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated saponins	15
3.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated sterols.	25
4.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated propanoic acid derivatives	27
5.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated lignans	29
6.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated cyanogenic compounds	30
7.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated phenolic compounds	31
8.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated cerebrosides	33
9.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated polyacetylenes	34
10.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated pyrrolidine derivatives	35
11.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated volatile oxygenated hydrocarbons	36
12.	Structures and distribution of secondary metabolites of <i>Polyscias</i> : chemical	
	structures of isolated monoterpenes	37
13.	Structures and distribution of secondary metabolites of Polyscias: chemical	
	structures of isolated sesquiterpenes	38
14.	Results of phytochemical screening of Polyscias guilfoylei and Polyscias	
	balfouriana leaves (Araliaceae) cultivated in Egypt	64
15.	Identification of the secondary metabolites predominant in the n -hexane fraction	
	of P. guilfoylei leaves by LC-ESI-MS	122

16.	Identification of the secondary metabolites predominant in the methylene						
	chloride fraction of <i>P. guilfoylei</i> leaves by LC-ESI-MS						
17.	Identification of the secondary metabolites predominant in the butanol fraction						
	of P. guilfoylei leaves by LC-ESI-MS	130					
18.	Mean inhibition zones of P. guilfoylei extract (PGE), fractions and compounds						
	4 & 9 against different ranges of environmental and clinically pathogenic						
	microorganisms determined by the agar well diffusion						
	method	135					
19.	Minimum Inhibitory Concentrations (MIC) of P. guilfoylei extract (PGE),						
	fractions against different a range of environmental and clinically pathogenic						
	microorganisms determined by the agar diffusion method	136					
20.	IC_{50} values (µg/mL) for cytotoxic effect of PGE, fractions and compounds 4 &						
	9 on the growth of MCF-7 and HCT cancer cell	137					
21.	Essential oil composition of <i>Polyscias balfouriana</i> leaves (PBL) and <i>Polyscias</i>						
	guilfoylei leaves (PGL)	148					
22.	Mean inhibition zones of volatile constituents of P. guilfoylei leaves (PGL)						
	and P. balfouriana leaves (PBL) against different ranges of environmental and						
	clinically pathogenic microorganisms determined by the agar diffusion						
	method.	153					
23.	Minimum Inhibitory Concentrations (MIC) volatile constituents of <i>P</i> .						
	guilfoylei leaves (PGL) and P. balfouriana leaves (PBL) against different						
	ranges of environmental and clinically pathogenic microorganisms determined						
	by the agar diffusion method	154					

List of Figures

N	Figure					
0						
1.	Whole shrub of <i>P. guilfoylei</i>	. 44				
2.	Branch of P. guilfoylei	44				
3.	Whole shrub of <i>P. balfouriana</i>	44				
4.	Branch of <i>P</i> .	45				
	balfouriana					
5.	Scheme showing chromatographic fractionation of <i>P. guilfoylei</i>	63				
	extract					
6.	¹ HNMR spectrum of <i>ent</i> -labda-8(17), 13-diene-15, 18-diol	. 68				
7.	¹³ CNMR of spectrum of <i>ent</i> -labda-8(17), 13-diene-15, 18-diol	69				
8.	HSQC spectrum of ent-labda-8(17), 13-diene-15, 18-diol	70				
9.	HMBC spectrum of ent-labda-8(17), 13-diene-15, 18-diol	71				
10	COSY spectrum of ent-labda-8(17), 13-diene-15, 18-diol	72				
11	¹ HNMR spectrum of	75				
	stigmasterol					
12	APT spectrum of stigmasterol.	76				
13	¹ HNMR spectrum of spinasterol.	79				
14	APT spectrum of spinasterol	. 80				
15	HSQC spectrum of spinasterol	81				
16	HMBC spectrum of spinasterol.	82				
17	FTIR spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	. 85				
18	ESI-MS spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	86				
19	¹ HNMRspectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	87				
20	APT spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl)	88				
	palmitamide					
21	HSQC spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	89				
22	HMBC spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	90				
23	HMBC spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	91				
24	HMBC spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide	92				

2526	HMBC spectrum of N-(2-S)-(1, 3-dihydroxyoctadecan-2-yl) palmitamide 9900000000000000000000000000000						
20 27	¹ HNMR spectrum of panaxydiol						
28	HSQC spectrum of panaxydiol						
29	COSY spectrum of panaxydiol.						
30	¹ HNMR spectrum of stigmasta-5, 22-dien-3-O-β- D glucopyranoside 1						
31	APT spectrum of stigmasta-5, 22-dien-3-O-β- D glucopyranoside						
32	HSQC spectrum of stigmasta-5, 22-dien-3-O-β- D glucopyranoside						
33	¹ H NMR spectrum of (8Z)-2-(2 hydroxypentacosanoylamino) octadeca-8-ene-1, 3, 4-triol						
34	APT spectrum of (8Z)-2-(2 hydroxypentacosanoylamino) octadeca-8-ene-1, 3, 4-triol						
35	COSY spectrum of (8Z)-2-(2 hydroxypentacosanoylamino) octadeca-8-ene-1, 3, 4-triol						
36	HSQC spectrum of (8Z)-2-(2 hydroxypentacosanoylamino) octadeca-8-ene-1, 3, 4-triol						
37	ESI-MS spectrum of 4-hydroxybenzoic acid						
38	¹ HNMR spectrum of 4-hydroxy benzoic acid						
39	APT spectrum of 4-hydroxy benzoic acid						
40	ESI-MS spectrum of tamaraxetin 3, 7-di- <i>O</i> -α-L-rhamnopyranoside						

41	¹ HNMR spectrum of tamaraxetin 3, 7-di- <i>O</i> -α-L-rhamnopyranoside	11
42	¹³ CNMR spectrum of tamaraxetin 3, 7-di- O -α-L-rhamnopyranoside	7 11
43	HSQC spectrum of tamaraxetin 3, 7-di- <i>O</i> -α-L-rhamnopyranoside	8 11
44	HMBC spectrum of tamaraxetin 3, 7-di-O-α-L-rhamnopyranoside	9 12
45	LC-MS chromatogram of <i>P. guilfoylei n</i> -hexane fraction	0 12
46	Chemical structures of compounds isolated from <i>P. guilfoylei n</i> -hexane fraction	3 12
47	LC-MS chromatogram of <i>P. guilfoylei</i> DCM fraction	4 12
48	Chemical structures of compounds identified from P. guilfoylei DCM fraction by	7
•	LC-MS	12 8
49	LC-MS chromatogram of <i>P. guilfoylei</i> butanol fraction	13 1
50	Chemical structures of compounds identified from <i>P. guilfoylei</i> butanol fraction by LC-MS	13 2
51	Inhibition % of histamine release by PGE	13 8
52	Inhibition % of histamine release by DCM fraction	13 9
53	Inhibition % of histamine release by EtOAc fraction	13 9
54	Inhibition % of histamine release by butanol fraction	14 0
55	Inhibition % of histamine release by N-(1, 3-dihydroxyoctadecan-2-yl) palmitamide (compound 4)	14

56	Inhibition	%	of	histamine	release	by	tamaraxetin	3,	7-di- O - α -L-	
	rhamnopyr	anosi	ide (compound 9)			· • • • • •		14
57	Inhibition	% of	colla	agenase by P	GE					1 14
•										2
58	GLC-profile obtained with a Rtx-5MS column of the essential oils isolated by									
	hydrodistillation for 3 h from A) P. guilfoylei and B) P. balfouriana								••••	14
										7

List of Abbreviations

Absorbance

)VA One-way analysis of variance

Attached proton test

C American type culture collection

Column chromatography

l₃ Deuterated chloroform

Centimeter

NMR Carbon-13 Nuclear Magnetic Resonance

Central nervous system

• Concentrated

Doublet

I Dichloromethane

Doublet of doublet

Diluted

SO- d_6 Deuterated dimethylsulfoxide- d_6

VMR Two-dimensional nuclear magnetic resonance spectroscopy

SA Enzyme-Linked Immunosorbent Assay

MS Electro-Spray Ionization Mass Spectrometry

Ac Ethyl acetate

Equatorial

Figure

Gram

ta. Gentamicin

Liquid Chromatography-Mass Spectrometry

Hours

Hydrochloric acid

-COSY H,H Correlated spectroscopy

BC Heteronuclear Multiple-Bond Correlation Spectroscopy

IMR Proton Nuclear Magnetic Resonance

HPLC High Performance Liquid Chromatography

HSQC Heteronuclear Single Quantum Coherence

Hz Hertz

IC₅₀ The half maximal inhibitory concentration

J Coupling constant

Keto. Ketoconazole

Kg Kilogram

KI Kovats index

L Liter

LC/MS Liquid Chromatography-Mass Spectroscopy

m Multiplet

MeOH Methanol

mg Milligram

mg/mL Milligram per milliliter

MHz Mega hertz

MIC Minimum inhibitory concentration

Min Minute

mL Milliliter

mm Millimeter

MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

m/z Mass to charge ratio

NA No activity

NT Not tested

p pentet

PGE Polyscias guilfoylei extract

ppm Part per million

PTLC Preparative thin layer chromatography

q Quartet

R_f Retardation factor

RI Retention index

ROS Reactive oxygen species

RPMI Roswell Park Memorial Institute (culture medium)

Retention time

s Singlet

S.D Standard deviation

Triplet

TLC Thin Layer Chromatography

TMS Tetramethylsilane

UV Ultraviolet

v/v Volume per volume

μg Microgram

μg/mL Microgram per milliliter

Introduction

Throughout ages, people have resorted to nature, mostly to plants as medical and health care sources for treatment and prevention of many diseases. In recent years, there has been a great use of plant based drugs in developing and developed countries. These drugs are derived from different plant parts. Plant derived natural products hold great hope for drug discovery. Nowadays, there is great increase in medicinal plant based industries and development of new drug molecules from natural products (Paarakh, 2010).

Medicinal plants and natural products have been reported to have various pharmacological activities; antimicrobial (Weckesser *et al.*, 2007), antioxidant (Katalinic *et al.*, 2006), hepatoprotective, hypoglycemic (Singab *et al.*, 2005), cytotoxic (Nibret *et al.*, 2010), estrogenic (Ashmawy *et al.*, 2016), antihypertensive (Braga *et al.*, 2000). These strong pharmacological activities of plants are contributed to their content of secondary metabolites that's why the medicinal plants are the object of biotechnologists for discovering new medicine (Marczewska *et al.*, 2011).

Family Araliaceae is a large family with 43 genera and 1400 species, which are widely used in traditional medicine and phytotherapy. The family is well known for its different classes of secondary metabolites such as triterpenoids, triterpenoidal saponins, diterpenes, sterols, acetylenic lipids, cerebrosides with anti-inflammatory, anti-proliferative, antidiabetic and anti-parasitic, CNS and CVS activities (A Clement and SH Clement, 2014).

Genus *Polyscias* comprises 116 species that are widely used as ornamental plants in addition to its various medicinal purposes mainly as anti-inflammatory, antitoxin, antibacterial and diuretic (Vo *et al.*, 1998).

Plants of this genus are widely cultivated in southeastern Asia and the tropical islands of the Pacific region. In Asian countries, the leaves are used as a tonic, anti-inflammatory, antitoxin, antibacterial, and are good for digestion. The

root is also used as a diuretic, febrifuge, anti-dysentery, and is employed for neuralgia and rheumatic pains (Huan *et al.*, 1997).

To our knowledge, only few reports are available in the current literature about the chemical constituents and the biological activities of the leaves of *Polyscias guilfoylei*. It was therefore, found interesting to subject the extract of leaves of entitled plant to a biological and phytochemical investigations.

Work strategy

- Collection, identification and authentication of the plant material; Polyscias guilfoylei and Polyscias balfouriana cultivated in Egypt.
- Preliminary phytochemical screening for Polyscias guilfoylei and Polyscias balfouriana leaves then phytochemical investigation of Polyscias guilfoylei leaves methanol extract to fractionate and separate different compounds
- Identification of the different isolated compounds using both chemical and spectroscopic methods of analysis including; IR, UV, ¹H-NMR and ¹³C-NMR.
- Evaluation of the biological activities of the methanolic extract of
 Polyscias guilfoylei leaves, different fractions and compounds, including
 mainly; antibacterial, antifungal, cytotoxic and histamine release
 inhibition activities.