



Phytochemical and biological investigations of *Coccoloba uvifera*, L. (Polygonaceae)

Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(Pharmacognosy)

By

Fatma Abdel Hakim Mohamed Ahmed

Bachelor of Pharmaceutical Sciences, 2013
Teaching assistant, Pharmacognosy and Medicinal Plants
Department
Faculty of Pharmacy, Heliopolis University for Sustainable
Development

**Cairo- Egypt
2020**



**Phytochemical and biological investigations of *Coccoloba uvifera*, L.
(Polygonaceae)**

Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree
In Pharmaceutical Sciences
(Pharmacognosy)

By

Fatma Abdel Hakim Mohamed Ahmed

Teaching Assistant, Pharmacognosy and Medicinal Plants Department
Faculty of Pharmacy, Heliopolis University for Sustainable
Development

Under Supervision of

Prof. Nahla A. Ayoub, PhD

Professor of Pharmacognosy,
Faculty of Pharmacy, Ain Shams University

Assoc. Prof. Mohamed Mahmoud El Shazly, PhD

Associate Professor of Pharmacognosy,
Faculty of Pharmacy, Ain Shams University

Assoc. Prof. Haidy Abdel Moniem Gad, PhD

Associate Professor of Pharmacognosy,
Faculty of Pharmacy, Ain-Shams University

**Cairo-Egypt
2020**

Acknowledgments

Firstly, thanks to **ALLAH** who has granted me many graces and blessed me by his generosity and mercy to accomplish this work.

It is a pleasure to express my deepest gratitude, sincere thanks and profound appreciation to **Prof. Dr. Nahla A. Ayoub** for setting an example to what a sincere professor, scientist, and advisor should be. I'd like to express my deep gratitude to her for supervising this study. **Assoc. Prof. Mohamed El-Shazly** for his kind help, valuable scientific advice, and great efforts throughout this work. **Assoc. Prof. Haidy Gad** for her continuous support, advice, and her kind help and supervision. **Dr. Reham El-Sharawy**, Lecturer of Pharmacognosy, Department of Phytochemistry and Plant Systematics, National Research Centre, for her supervising and following up the practical work, suggesting the titled plant, and for her continuous encouragement. **Dr. Rasha Ali Radwan**, Lecturer of Biochemistry, Department of Biochemistry, Sinai University-Kantara branch, for her efforts, support, assistance in the biological assays and revising the thesis.

I would like to express my profound gratitude to my professors and colleagues from the Department of Pharmacognosy and Medicinal Plants, Heliopolis University for Sustainable Development, especially Dr. Mouchira Choukry, Dr. Mohamed Nabil, Dr. Sameh Fikry for their cooperation, kind advice and support.

Special thanks and debt of gratitude to my parents for their continuous support, encouragement, help, sacrifices, and prayers throughout my whole life, also I would like to thank my brother and my family for their support and encouragement.

والحمد لله رب العالمين.....

Fatma Abdel Hakim Mohamed Ahmed

2020

Table of Contents

Table of Contents	I
List of Abbreviations	IV
List of Figures	VIII
List of Tables	X
Abstract	XII
Introduction	1
Taxonomy	4
Review of literature	7
1. Biological activities of the genus <i>Coccoloba</i>	8
2. Phytochemical constituents of the genus <i>Coccoloba</i>	16
Materials, Methods and Apparatus	28
1. Plant material.....	28
2. Material for the phytochemical investigation of the leaves of <i>Coccoloba uvifera</i> L.	28
3. Materials for the biological assays of the aqueous ethanolic extract of the leaves of <i>Coccoloba uvifera</i> , L.....	32
5. Methods.....	39
4. Apparatus.....	45

Results and Discussion

Chapter (1): Phytochemical investigation of the leaves of *Coccoloba uvifera* L.

.....46

1.1. Phytochemical screening of *Coccoloba uvifera* leaves.....46

1.2. Fractionation of CLE using column chromatography.....47

1.3. Isolation and identification of CLE compounds (1-7).....48

1.3.1. Fraction II50

1.3.1.1. Identification of compound (1); Protocatechuic acid.....50

1.3.1.2. Identification of compound (2); Gallic acid.....55

1.3.2. Fraction III.....59

1.3.2.1. Identification of compound (3); Myricetin-3-O-rhamnoside59

1.3.2.2. Identification of compound (4); Quercetin 3-O- α -rhamnoside.....64

1.3.3. Fraction IV.....67

1.3.3.1. Identification of compound (5); Quercetin-3-O-glucoside.....67

1.3.4. Fraction V.....72

1.3.4.1. Identification of compound (6); Quercetin.....72

Chapter (2): Biological assays of aqueous ethanolic leaves extract of *Coccoloba uvifera* L.....76

2.1. Anti-diabetic activity (α -amylase inhibition assay)76

2.2. Anti-obesity activity (Pancreatic lipase inhibition assay).....78

Table of Contents

2.3. Anti-ageing activity assays	81
2.3.1. Collagenase inhibition assay	81
2.3.2. Elastase inhibition assay	84
2.4. Skin whitening activity	86
2.5. Anti- inflammatory assays.....	88
2.5.1. Superoxide generation inhibition.....	89
2.5.2. Elastase release inhibition assay.....	91
General summary.....	93
Conclusion and recommendations.....	98
References	100
Arabic summary	

List of Abbreviations

¹ H-NMR: ¹H Nuclear Magnetic Resonance

¹³ C-NMR: ¹³C Nuclear Magnetic Resonance

AAPH: 2,2'-azobis 2 amidino propane dihydrochloride

ABTS: 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)

AchE: Acetyl Cholinesterase

Amm.: Ammonia

α-MSH: α-Melanocyte-stimulating hormone

BAW: Butanol:Acetic acid:Water

BSLA: Brine shrimp lethal assay

BuChE: Butyrylcholinesterase

BuOH: Butanol

CB: Cytochalasin B

CC: Column chromatography

CCL₂: The chemokine (C-C motif) ligand 2

CoPC: Comparative Paper Chromatography

CLE: *Coccoloba uvifera* leaves extract

d: Doublet

List of Abbreviations

dd: Doublet of doublets

DMSO-d₆: Deuterated dimethylsulfoxide

DNS: Dinitrosalicylic acid

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

ECM: Extracellular matrix

EDTA: Ethylene diamine tetraacetate

EtOH: Ethyl alcohol

FALGPA: N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala

FMLF: Formyl-methionyl-leucyl-phenylalanine

g: Gram

h: Hour(s)

H₃BO₃: Boric acid

HOAc-6: 6% Acetic acid

IC₅₀: The half-maximal inhibitory concentration

IL-1 α : Interleukin 1alpha

J value: Coupling constant

LD₅₀: The amount of the substance required to kill 50% of the test population

L-DOPA: L-3,4-Dihydroxyphenylalanine

LNCaP: Prostate cancer androgen-sensitive cell line

LPS: Lipopolysaccharide

List of Abbreviations

M.wt. Molecular weight

m: Meter

MeOH: Methanol

MIC: Minimum inhibitory concentration

ml: Milliliter

mm: Millimeter

mM: Millimole

MMPs: Matrix metalloproteinases

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

NaOAc: Sodium acetate

NMR: Nuclear magnetic resonance spectroscopy

OD550: Optical density at wavelength 550 nm

PC: Paper Chromatography

PCa: Prostate cancer

PL: Pancreatic lipase

PPL: Porcine pancreatic lipase

ppm: Part per million

Prep. PC: Preparative paper chromatography

Rev/ug: Revertants/ug

R_f: Retention factor

List of Abbreviations

ROS: Reactive oxygen species

rpm: Revolutions per minute

s: Singlet

SD: Standard deviation

S.E.M.: Standard error of the mean

t: Triplet

TES: Tris(hydroxymethyl)-methyl-2-aminoethane sulfonate

THP-1: human acute monocytic leukemia cell line

TLC: Thin layer chromatography

TMS: Tetramethylsilane

TNF- α : Tumor necrotic factor- α

UV: Ultraviolet

δ : Chemical shift

λ : Wavelength

μ : Micro

LIST OF FIGURES

Fig. (1): Photo of <i>Coccoloba uvifera</i> L.	29
Fig. (2): Scheme for the extraction of <i>Coccoloba uvifera</i> leaves and isolation of its components.	49
Fig. (3): ¹H NMR spectrum of compound (1): Protocatechuic acid.	53
Fig. (4): ¹³C NMR spectrum of compound (1): Protocatechuic acid.	54
Fig. (5): ¹H-NMR spectrum of compound (2): Gallic acid.	57
Fig. (6): ¹³C spectrum of compound (2): Gallic acid.	58
Fig. (7): ¹H-NMR spectrum of compound (3): Myricetin-3-O-rhamnoside (myricitrin).	62
Fig. (8): ¹³C spectrum of compound (3): Myricetin-3-O-rhamnoside (myricitrin).	63
Fig. (9): ¹H spectrum of compound (4): Quercetin-3-O-α-rhamnoside (quercitrin).	66
Fig. (10): ¹H-NMR Spectrum of compound (5): Quercetin-3-O-glucoside.	70
Fig. (11): ¹³C-NMR Spectrum of compound (5): Quercetin-3-O-glucoside.	71
Fig. (12): ¹H-NMR Spectrum of compound (6): Quercetin.	74
Fig. (13): ¹³C-NMR Spectrum of compound (6): Quercetin.	75
Fig. (14): α-Amylase inhibition percentage of <i>Coccoloba uvifera</i> extract, myricitrin and quercetin at 300 μg/ml.	78

List of Figures

Fig. (15): Pancreatic lipase inhibition of *Coccoloba uvifera* extract, myricitrin and quercetin at 100 µg/ml. 81

Fig. (16): Collagenase inhibition of *Coccoloba uvifera* extract, myricitrin and quercetin at 500 µg/ml. 83

Fig. (17): Elastase inhibition of *Coccoloba uvifera* extract, myricitrin and quercetin at 300 µg/ml. 85

Fig. (18): Tyrosinase inhibition of *Coccoloba uvifera* extract, myricitrin and quercetin at 300 µg/ml. 88

List of Tables

Table (1): Flavonoids belonging to the genus <i>Coccoloba</i> .	15
Table (2): Sterols belonging to the genus <i>Coccoloba</i> .	17
Table (3): Triterpenes belonging to the genus <i>Coccoloba</i> .	19
Table (4): Tannins belonging to the genus <i>Coccoloba</i> .	22
Table (5): Diterpenes belonging to the genus <i>Coccoloba</i> .	22
Table (6): Phenolic acids belonging to the genus <i>Coccoloba</i> .	23
Table (7): Anthraquinones belonging to the genus <i>Coccoloba</i> .	24
Table (8): Isochromene belonging to the genus <i>Coccoloba</i> .	26
Table (9): Solvent systems for paper chromatography.	33
Table (10): Results of phytochemical screening of <i>Coccoloba uvifera</i> dried leaves.	47
Table (11): Results of column fractionation of CLE.	48
Table (12): Chromatographic and spectroscopic data of compound (1).	52
Table (13): Chromatographic and spectroscopic data of compound (2).	56
Table (14): Chromatographic and spectroscopic data of compound (3).	61
Table (15): Chromatographic and spectroscopic data of compound (4).	65
Table (16): Chromatographic and spectroscopic data of compound (5).	69
Table (17): Chromatographic and spectroscopic data of compound (6).	73
Table (18): α -amylase percentage inhibition of <i>Coccoloba uvifera</i> extract, myricitrin, quercetin at 300 $\mu\text{g/ml}$ (Mean $\pm$ S.D.) and their IC_{50} ($\text{IC}_{50} \pm$ S.D.).	77

List of Tables

Table (19): Pancreatic lipase inhibition of <i>Coccoloba uvifera</i> extract, myricitrin and quercetin at 100 µg/ml (Mean ± S.D.) and their IC ₅₀ (IC ₅₀ ± S.D.).	80
Table (20): Collagenase inhibition of <i>Coccoloba uvifera</i> extract, myricitrin and quercetin at 500 µg/ml (Mean ± S.D.) and their IC ₅₀ (IC ₅₀ ± S.D.).	83
Table (21): Elastase inhibition of <i>Coccoloba uvifera</i> extract, myricitrin and quercetin at 300 µg/ml (Mean ± S.D.) and their IC ₅₀ (IC ₅₀ ± S.D.).	85
Table (22): Tyrosinase inhibition of <i>Coccoloba uvifera</i> extract, myricitrin and quercetin at 300 µg/ml (Mean ± S.D.) and their IC ₅₀ (IC ₅₀ ± S.D.).	87
Table (23): Superoxide generation inhibition of <i>Coccoloba uvifera</i> extract, isolated compounds and different fractions at 10 ug/ml (Mean ± S.E.M) and their IC ₅₀ (IC ₅₀ ± S.E.M).	90
Table (24): Elastase release inhibition of <i>Coccoloba uvifera</i> extract, isolated compounds and different fractions at 10 ug/ml (Mean ± S.E.M) and their IC ₅₀ (IC ₅₀ ± S.E.M).	92

Abstract

Phytochemical and biological investigations of *Coccoloba uvifera* L.

(Polygonaceae).

Key Words: *Coccoloba uvifera*, Polygonaceae, anti-diabetic, anti-obesity, anti-aging, anti-inflammatory.

Six compounds were isolated from the aqueous ethanolic leaves extract of *Coccoloba uvifera* L. The structures of the compounds were identified on the basis of spectroscopic data UV, ^1H -NMR, ^{13}C -NMR. Also, biological investigations were done on the extract and the isolated compounds, which showed a significant antidiabetic activity through α -amylase inhibition, anti-obesity activity through pancreatic lipase inhibition, anti-aging and anti-inflammatory activities.