

**Isolation, Structure Elucidation, Metabolite Profiling
and Bio-, Pharma- Activity Screening of
Eichhornia crassipes (Mart.) Solms**

**By
P. Jayanthi**

***Supervisor*
Dr. P. Lalitha**

**A thesis submitted to
Avinashilingam Institute for Home Science and
Higher Education for Women,
Coimbatore - 641043**

**In partial fulfilment of the requirement for the Degree of
Doctor of Philosophy in Chemistry**

December, 2012

CERTIFICATE

This is to certify that the dissertation entitled "**Isolation, Structure Elucidation, Metabolite Profiling and Bio-, Pharma- Activity Screening of *Eichhornia crassipes* (Mart.) Solms**" submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in partial fulfilment of the requirement for the award of the **Degree of Doctor of Philosophy in Chemistry** is a record of original research work done by **Ms. P. Jayanthi** during the period of her study in the Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or similar title to any candidate of any other University.


Dr. R. Shyamala, M.Sc., Dip.Ed., M.Phil, Ph.D
Professor & Head
Department of Chemistry
Avinashilingam Institute for Home
Science and Higher Education for Women,
Coimbatore - 641 043
Signature of the Head of the Department


Signature of the Guide


Signature of the Dean

Dr. R. PARVATHI, M.Sc., M.Phil, Ph.D.
Dean, Faculty of Science
Professor & Head
Dept of Biochemistry & Biotechnology
Avinashilingam Institute for Home Science
and Higher Education for Women
Coimbatore - 641043

DECLARATION

I hereby declare that the thesis entitled "**Isolation, Structure Elucidation, Metabolite Profiling and Bio-, Pharma- Activity Screening of *Eichhornia crassipes* (Mart.) Solms**" submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in partial fulfilment of the requirement for the award of the **Degree of Doctor of Philosophy in Chemistry** is a record of original research work done by me under the supervision and guidance of **Dr. P. Lalitha, M.Sc., M.Phil., Ph.D., (Avinashilingam)**, Assistant Professor (SS), Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and it has not formed the basis for the award of any Degree/ Diploma/Associateship/Fellowship or similar title to any candidate of any other University.


Signature of the Guide


Signature of the Candidate

Contents

Chapter No.	Title	Page No.
	List of Tables	
	List of Figures	
	List of Abbreviations and Acronyms	
1.0	Introduction	1
2.0	Review of Literature	17
3.0	Materials and Methods.....	52
4.0	Results and Discussion	77
5.0	Summary and Conclusion.....	210
	References and Bibliography	215
	Publications	

List of Tables

Table No.	Title	Page No
1	Instruments/ equipments used during the research	52
2	Comparison of different extraction methods for fresh <i>E. crassipes</i> with ethyl acetate	55
3	Nature of the fractions and polarity of the solvent in acetone column	59
4	Nature of the fractions and polarity of the solvent used in ethyl acetate column of <i>E. crassipes</i>	60
5	Nature of the fractions and polarity of the solvent used in aqueous column of <i>E. crassipes</i>	61
6	Nature of the fractions and the polarity of the solvent used in petroleum ether fractionate column	63
7	Constituents of the skin cream	71
8	Yield (g) of extracts obtained in different extraction methods for dried <i>E. crassipes</i> (3 h)	78
9	Yield of the extract obtained by conventional and sound assisted extractions for fresh <i>E. crassipes</i>	79
10	Time and yield of ten different methods of extraction of fresh <i>E. crassipes</i> with ethyl acetate	81
11	Preliminary phytochemical screening of the solvent extracts and solvent fractionates of aqueous extracts of <i>E. crassipes</i>	89
12	Phytochemicals in various extracts of <i>E. crassipes</i>	88
13	¹ H NMR, ¹³ C NMR, DEPT 135, ¹ H- ¹ H COSY and HMBC data of A15	98
14	¹ H NMR, ¹³ C NMR, DEPT 45, DEPT 90, DEPT 135, ¹ H- ¹ H COSY and HMBC data of A20	104
15	¹ H NMR, ¹³ C NMR, DEPT 90, ¹ H- ¹ H and HMBC data of A22	107
16	¹ H NMR, ¹³ C NMR, DEPT 45, DEPT 135 spectral values, ¹ H- ¹ H COSY and HMBC correlation of E6	115
17	¹ H NMR, ¹³ C NMR, DEPT 45, DEPT 90 and DEPT 135 spectral values, ¹ H- ¹ H COSY and HMBC correlation of E8	120

Table No.	Title	Page No
18	^1H NMR, ^{13}C NMR, DEPT 90, DEPT 135 values, ^1H - ^1H COSY and HMBC data of E10	123
19	^1H NMR and ^{13}C NMR values of E11	134
20	^1H NMR and ^{13}C NMR values of E12	137
21	^1H NMR and ^{13}C NMR values of E13	140
22	Yield of stigmasterol isolated from various plants	142
23	^1H NMR, ^{13}C NMR values, ^1H - ^1H COSY and HMBC correlation of E16	147
24	^1H NMR, ^{13}C NMR values and HMBC correlation of E21	154
25	^1H NMR, ^{13}C NMR values, ^1H - ^1H COSY and HMBC correlation of EAC1	161
26	^1H NMR, ^{13}C NMR values, ^1H - ^1H COSY and HMBC correlation of EAC2	165
27	^1H NMR, ^{13}C NMR, DEPT 45, DEPT 90, DEPT 135 values, ^1H - ^1H COSY and HMBC correlations of HP1	169
28	^1H NMR, ^{13}C NMR, DEPT 135 values, ^1H - ^1H COSY and HMBC correlation of HP6	173
29	Compounds isolated from various extracts of <i>E. crassipes</i> in the present study	179
30	Antibacterial activity of the extracts/ fractionates and E12 of <i>E. crassipes</i>	183
31	Antifungal activity of the extracts/ fractionates and E12 of <i>E. crassipes</i>	185
32	Reducing capacity of the extracts/ fractionates of <i>E. crassipes</i> as a function of concentration	187
33	Reducing capacity of the extracts/ fractionates of <i>E. crassipes</i> as a function of time at 50 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ for AFM	187
34	DPPH radical scavenging activity of the extracts and fractionates of <i>E. crassipes</i>	189
35	Effect of EA, AQ and MFA of <i>E. crassipes</i> on acute oral toxicity test in mice	191

Table No.	Title	Page No
36	Dosage of <i>E. crassipes</i> extracts administered to test animals	192
37	Effect of the ointments on wound area (mm ²) in rats	194
38	Effect of the ointments on rate of wound contraction in rats	194
39	Physico chemical parameters of the skin cream	199
40	Percentage inhibition of DNA damage of LPR1 and LP3	201
41	DPPH radical scavenging activity of LPR1 and LP3	201
42	Toxicity of extracts/ fractionates of <i>E. crassipes</i> on larvae (I, II, III and IV) and pupae of <i>C. quinquefasciatus</i> at 24 h of exposure	203
43	Larvicidal and Pupicidal activity of extracts/ fractionates of <i>E. crassipes</i> against <i>C. quinquefasciatus</i>	205
44	Docking energy score of ligands against the receptors COX-1, COX-2 and SIRT1	208

List of Figures

Figure No.	Title	Page No
1	Gas chromatogram of sterols isolated from <i>E. crassipes</i>	83
2	Gas Chromatogram of alkaloids isolated from methanol fractionate of aqueous extract of <i>E. crassipes</i>	84
3	Gas Chromatogram of terpenoids isolated from ethyl acetate extract of <i>E. crassipes</i>	85
4	UV spectrum of A5	92
5	IR spectrum of A5	92
6	¹ H NMR spectrum of A5	93
7a	¹³ C NMR spectrum of A5	94
7b	DEPT 45 spectrum of A5	94
7c	DEPT 135 spectrum of A5	94
8	¹ H- ¹ H COSY spectrum of A5	95
9	HSQC spectrum of A5	95
10	¹ H NMR spectrum of A15	99
11	¹³ C NMR spectrum of A15	100
12	¹ H- ¹ H COSY spectrum of A15	101
13	HSQC spectrum of A15	101
14	HMBC spectrum of A15	102
15	¹ H- ¹ H COSY and HMBC correlations of A15	102
16	HMBC and ¹ H- ¹ H COSY correlations of A20	105
17	¹ H NMR spectrum of A22	109
18	¹³ C NMR spectrum of A22	110
19	DEPT 45 spectrum of A22	111
20	DEPT 135 spectrum of A22	111

Figure No.	Title	Page No
21	^1H - ^1H COSY spectrum of A22	112
22	HSQC Spectrum of A22	112
23	HMBC spectrum of A22	113
24	^1H NMR spectrum of E6	116
25	^{13}C NMR spectrum of E6	116
26	^1H - ^1H COSY spectrum of E6	117
27	HSQC spectrum of E6	117
28	HMBC spectrum of E6	118
29	^1H NMR spectrum of E8	121
30	^{13}C NMR spectrum of E8	121
31	UV spectrum of E10	126
32	IR spectrum of E10	126
33	^1H NMR spectrum of E10	127
34	^{13}C NMR spectrum of E10	128
35	DEPT 45 NMR spectrum of E10	129
36	^1H - ^1H COSY spectrum of E10	129
37	^1H - ^{13}C HSQC spectrum of E10	130
38	^1H - ^{13}C HMBC spectrum of E10	130
39	^1H - ^1H COSY and HMBC correlations of hexadecanyl 2-hydroxy 4-methoxy cinnamate	131
40	Distinguishable ^1H - ^1H COSY and HMBC correlations of campesterol	133
41	HPTLC chromatogram of E11	133
42	Key HMBC correlation of β -sitosterol	137
43	Key HMBC and ^1H - ^1H COSY of stigmasterol	141

Figure No.	Title	Page No
44	UV spectrum of E16	146
45	IR spectrum of E16	146
46	^1H NMR spectrum of E16	148
47	^{13}C NMR spectrum of E16	149
48	DEPT 45 spectrum of E16	150
49	DEPT 90 spectrum of E16	150
50	DEPT 135 spectrum of E16	151
51	^1H - ^1H COSY spectrum of E16	151
52	HSQC spectrum of E16	152
53	HMBC spectrum of E16	152
54	HMBC and ^1H - ^1H COSY correlations of E16	153
55	^1H NMR spectrum of E21	155
56	^{13}C NMR spectrum of E21	155
57	^1H - ^1H COSY spectrum of E21	156
58	HSQC spectrum of E21	156
59	HMBC spectrum of E21	157
60	^1H NMR spectrum of W5	159
61	^1H - ^1H COSY spectrum of W5	158
62	^1H NMR spectrum of EAC1	162
63	^{13}C NMR spectrum of EAC1	163
64	HSQC spectrum of EAC1	164
65	^1H NMR spectra of EAC2	166
66	^{13}C NMR spectra of EAC2	167
67	^1H NMR spectrum of HP1	170

Figure No.	Title	Page No
68	¹³ C NMR spectrum of HP1	171
69	¹ H NMR spectrum of HP6	174
70	¹³ C NMR spectrum of HP6	175
71	DEPT 45 spectrum of HP6	176
72	¹ H- ¹ H COSY spectrum of HP6	176
73	HSQC spectrum of HP6	177
74	HMBC spectrum of HP6	177
75	HPLC of the ethyl acetate extract of <i>E. crassipes</i>	181
76	HPLC of the aqueous extract of <i>E. crassipes</i>	181
77	Plates depicting the antimicrobial activity of <i>E. crassipes</i>	184
78	Photograph of Swiss Albino mice used in acute oral toxicity studies	190
79	Wound healing activity of extract of <i>E. crassipes</i> in Albino Wistar rats	193
80	Effect of the ointments and formulation on the tensile strength of the animals	195
81	Photomicrographs of sections of the skin from treated rats stained with hematoxylin and eosin. Skin microscopic image of F1, F2, F3, F4, F5, F6 and F7	197
82	Percentage mortality of <i>Culex quinquefasciatus</i> larvae on treatment with extracts and fractionates of <i>E. crassipes</i>	202
83	Percentage mortality of <i>Culex quinquefasciatus</i> pupae on treatment with extracts and fractionates of <i>E. crassipes</i>	204
84	Ligands chosen for the molecular docking	207
85	Representative docking of the ligand stigma 5,22-diene-3,7-diol to the receptor COX-1	208

Abbreviations and Acronyms

kg	Kilogram
g	gram
µg	Milligram
mg	Microgram
m ²	Meter square
cm	Centimeter
nm	Nanometer
µM	Micrometer
mM	Milli Molar
mol	Mole
°C	Degree Celsius
h	Hour
L	Litre
mL	Millilitre
U/mL	Units per Milliliters
ppt	parts per trillion
ppm	parts per million
eV	electron Volt
GAE	Gallic Acid Equivalents
body wt.	Body weight
TLC	Thin Layer Chromatography
CC	Column Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
LC-MS	Liquid Chromatography - Mass Spectrometry
PDA	Photoelectron Diode Array
UV	Ultra Violet
FTIR	Fourier Transform Infrared Spectroscopy
¹ H NMR	Proton Nuclear Magnetic Resonance
¹³ C NMR	Carbon Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer

¹ H - ¹ H COSY	Proton - Proton COrrelation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
AA	Ascorbic Acid
TFA	Trifluoroacetic acid
TCA	Trichloro acetic acid
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
DPPH	1, 1 Diphenyl 2, picryl hydrazyl
ABTS	2, 2-Azinobis (3-ethyl benzothiazoline- 6- sulfonic acid)
DMPD	N, N-Dimethyl-p-phenylene diamine dihydrochloride
ORAC	Oxygen Radical Absorbance Capacity
FRAP	Ferric Reducing Ability of Plasma
TRAP	Total Radical Trapping Antioxidant Parameter
HMDB	Human Metabolome Database
NSAIDs	Nonsteroidal anti-inflammatory drugs
CoMFA	Comparative Molecular Field Analysis
CoMSIA	Comparative Molecular Similarity Indices Analysis
SOMFA	Self-Organizing Molecular Field Analysis
ANOVA	ANalysis Of VAriance
COX	Cyclooxygenase
SIRT	Sirtuin
PG	Prostaglandin
PDB	Protein Data Bank
HTS	High Throughput Screening
VS	Virtual Screening
GLIDE	Grid Based Ligand Docking with Energetics
SLIDE	Screening for Ligands by Induced-fit Docking
pBR322	Plasmid Bolivar and Rodriguez 322
pTZ57R/T	T-vector containing cDNA
PHF	Polyherbal Formulation
LCL	Lower Confidence Limit
UCL	Upper Confidence Limit
WSSA	Weed Science Society of America
EPA	Environmental Protection Agency
