

Acknowledgement

ACKNOWLEDGEMENT

I thank the Lord almighty, for His constant shower of blessings to complete my work successfully.

*I express my deep sense of gratitude to our Chairperson **Smt. Dr. R. Nandhini**, PSGR Krishnammal College for women, Coimbatore, for providing me the opportunity to pursue my studies in this esteemed institution.*

*I owe my sincere thanks to **Dr. (Mrs.) Nirmala Devi**, Principal, PSGR Krishnammal College for women, for providing me with all the facilities to carry out my research work successfully.*

*I am grateful to **Mrs. S. Vasandha, M.Sc., M.Phil.** former head, Department of Botany and Dean, student affairs, for her support and encouragement throughout the course of study.*

*I express my sincere thanks to former Heads of the department **Dr. V. Sashi, Dr. S. Meerabai and Dr. S. N. Padma Devi** for their constant support and encouragement.*

*I express my gratefulness to **Dr. C. Krishnaveni. M.S.c, M.Phil., B.Ed., Ph.D.**, Head, Department of Botany, PSGR Krishnammal College for women, Coimbatore for her constant support and encouragement throughout the course of the Research.*

*I place my heartfelt gratitude to my guide, **Dr. M. Kamalam, M.Sc., M.Phil., Ph.D.**, Associate professor, Department of Botany, PSGR Krishnammal college for women, Coimbatore for her valuable suggestions, guidance, constant support, encouragement, supervision and care throughout the course and for the help in completing the research work successfully.*

*My special thanks to **Dr. Marie Josphine, M.Sc., M.Phil., Ph.D., PGDCA Associate Professor, Nirmala College for Women** for her valuable suggestions during Doctoral committee visit.*

*I express my thanks to **Dr. M. Ramanthan, M. Pharm., Ph.D** and **Dr. G. Syamala, M. Pharm., Ph.D** PSG college of Pharmacy, Coimbatore for their help to carry over the HPTLC analysis.*

*I express my thanks to **Dr.G.Ariharasivakumar, M. Pharm., Ph.D** KMCH College of Pharmacy, Coimbatore for permitting me to conduct the Cell line studies.*

*My sincere and special gratitude to **Dr. U. Umadevi, Department of Botany, SFR College for Women, Sivakasi** for her help and encouragement.*

*I thank my friends **Mrs. S. Rukshana Begum, Mrs. P. Kemila, Dr. S. Kalaiarasi, Mrs. P.R. Sowmiya, Ms. P. Uma Maheswari** and all the Research Scholars in Botany Department, for their help rendered throughout this research programme.*

*I owe my sincere thanks to all the **Faculty members** of Department of Botany, for their help rendered whenever necessary.*

*I express my thanks to all **Non-teaching staff** of Department of Botany, PSGR Krishnammal College for women, Coimbatore for their assistance throughout the Research period.*

*With love and affection, I wish to express my deep sense of gratitude to my beloved Parents **Mr.K.S.Velusamy** and **Mrs.V. Suseela** and my brother **Mr.V. Vinoth** for their moral support and blessings. I extend my gratitude to my lovable husband **Mr.V. Gopalakrishnan** for his encouragement and support.*

Dedication



Dedicated to my Family

Contents

CONTENTS

S.No	Particulars	Page No
	List of Abbreviations used List of Tables List of Figures List of plates	
1	Introduction	1
2	Review of Literature	5
3	Materials and methods	40
4	Results	59
5	Discussion	138
6	Summary	156
7	Conclusion	160
8	References	i-iv
9	Annexure Publications	

List of Abbreviations

LIST OF ABBREVIATIONS

AAS	- Atomic Absorption Spectrophotometer
ACS	- American Cancer Society
AFLP	- Amplified fragment length polymorphism
ANOVA	- Analysis of variance
ATCC	- American Type Culture Collection
BA	- 6-Benzyladenine
BAP	- 6-Benylaminopurine
CTAB	- Cetyl Trimethyl Ammonium Bromide
DMEM	- Dulbecco's Modified Eagle Medium
DMSO	- Dimethyl sulfoxide
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
FBS	- Fetal Bovine Serum
FDA	- Food and Drug Administration
GA₃	- Gibberellic acid
GCMS	- Gas Chromatography and Mass spectrometry
HPTLC	- High Performance Thin Layer Chromatography
IAA	- Indole-3-acetic acid
IBA	- Indole-3-butyric acid
IR	- Infra Red
KN	- Kinetin
LDH	- Lactate dehydrogenase
MIC	- Minimum Inhibitory Concentration
MS	- Murashige and Skoog
MTT	- 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
NAA	- Naphthalene Acetic Acid

NADH	- Nicotine amide-adenine-dinucleotide
NCCAM	- National Center for Complementary and Alternative Medicine
NIST	- National Institute of Standard Technology
OPLC	- Optimum Performance Laminar Chromatography
PCR	- Polymerase chain reaction
PDA	- Potato Dextrose Agar
PPM	- Parts Per Million
RAPD	- Random Amplified Polymorphic DNA
RFLP	- Restriction Fragment Length Polymorphism
SPSS	- Statistical Package for the Social Sciences
SRB	- Sulforhodamine B
SSR	- Simple Sequence Repeats
TBE	- Tris/Borate/EDTA
TCA	- Trichloroacetic acid
TE	-Tris-EDTA
TLC	-Thin Layer Chromatography
2,4-D	- 2,4-Dichlorophenoxyacetic acid

List of Tables

LIST OF TABLES

Table No	Title	Page No
Chapter I Pharmacognostical studies		
1	Physicochemical analysis	59
2	Extractive values	60
3	Fluorescence analysis	62
4	Qualitative phytochemical studies on <i>E. glandulosum</i>	64
5	Qualitative phytochemical studies on <i>E. odoratum</i>	65
6	Qualitative phytochemical studies on <i>E. triplinerve</i>	66
7	GCMS analysis of leaf extract of <i>E. glandulosum</i>	69
8	GCMS analysis of leaf extract of <i>E. odoratum</i>	70
9	GCMS analysis of leaf extract of <i>E. triplinerve</i>	71
10	Quantitative estimation of quercetin content in the leaf extracts of selected plants using HPTLC	75
11	Heavy metal analysis	77
12	Antibacterial activity of leaf extract of <i>E. glandulosum</i>	79
13	Antibacterial activity of leaf extract of <i>E. odoratum</i>	80
14	Antibacterial activity of leaf extract of <i>E. triplinerve</i>	81
15	MIC studies of leaf extracts against bacterial pathogens	82
16	Antifungal activity of leaf extracts of <i>E. glandulosum</i>	85
17	Antifungal activity of leaf extract of <i>E. odoratum</i>	86
18	Antifungal activity of leaf extract of <i>E. triplinerve</i>	87

19	MIC studies of leaf extracts against fungal pathogens	88
20	Antioxidant activity of leaf extracts of selected plants using DPPH method	91
21	Antioxidant activity of leaf extracts of selected plants using Hydrogen peroxide scavenging method	92
22	RAPD banding patterns of selected <i>Eupatorium</i> species using different primers	96
23	<i>In vitro</i> cytotoxic effect of leaf extracts against human colon carcinoma (HT-29) by MTT assay	98
24	<i>In vitro</i> cytotoxic effect of leaf extracts against human colon carcinoma (HT-29) by SRB assay	99
25	<i>In vitro</i> cytotoxic effect of leaf extracts against human colon carcinoma (HT-29) by LDH assay	101
Chapter II Tissue culture studies		
26	Effect of 2,4-D on callus formation in leaf explants	104
27	Effect of 2,4-D on callus formation in internodal explants	106
28	Effect of 2,4-D on callus formation in nodal explants	108
29	Effect of factorial combination of IAA and BA on callus formation in leaf explants	110
30	Effect of factorial combination of IAA and BA on callus formation in internodal explants	112
31	Effect of factorial combination of IAA and BA on callus formation in nodal explants	114
32	Effect of factorial combinations of IAA and BA on shoot bud induction in nodal explants of <i>E. glandulosum</i>	116
33	Effect of plant growth regulators and seasonal differences on shoot induction in nodal explants of <i>E. glandulosum</i>	118
34	Induction of roots on <i>in vitro</i> grown shoots	120

35	Effect of various potting mixtures on the growth of <i>in vitro</i> grown plants of <i>E. glandulosum</i>	122
Chapter III Screening of <i>in vitro</i> grown materials for secondary metabolites		
36	GCMS analysis of callus extract of <i>E. glandulosum</i>	127
37	GCMS analysis of callus extract of <i>E. odoratum</i>	128
38	GCMS analysis of callus extract of <i>E. triplinerve</i>	129
39	GCMS analysis of leaf extract of hardened plants of <i>E. glandulosum</i>	131

List of Figures

LIST OF FIGURES

Figure No	Title	Page No
Chapter I Pharmacognostical studies		
1	GCMS analysis of leaf extract of <i>E. glandulosum</i>	68
2	GCMS analysis of leaf extract of <i>E. odoratum</i>	68
3	GCMS analysis of leaf extract of <i>E. triplinerve</i>	68
4	HPTLC analysis in leaf extracts of selected plants	73
5	HPTLC chromatogram of leaf extracts of selected plant	74
6	Quercetin content in the leaf extracts of selected plants	75
7	MIC studies of leaf extracts against bacterial pathogens	83
8	MIC studies of leaf extracts against fungal pathogens	89
9	Antioxidant activity of leaf extracts	93
10	RAPD amplification of selected <i>Eupatorium</i> species	95
11	<i>In vitro</i> cytotoxic effect of leaf extracts against HT-29 cell line (MTT assay)	102
12	<i>In vitro</i> cytotoxic effect of leaf extracts against HT-29 cell line (SRB assay)	102
13	<i>In vitro</i> cytotoxic effect of leaf extracts against HT-29 cell line (LDH assay)	102
Chapter II Tissue culture studies		
14	Effect of various potting mixtures on the growth of <i>E. glandulosum</i>	122
Chapter III Screening of <i>in vitro</i> grown materials for secondary metabolites		
15	GCMS analysis of callus extract of <i>E. glandulosum</i>	126
16	GCMS analysis of callus extract of <i>E. odoratum</i>	126
17	GCMS analysis of callus extract of <i>E. triplinerve</i>	126

18	GCMS analysis of leaf extract of hardened plants of <i>E. glandulosum</i>	130
19	HPTLC analysis of callus extract of selected plants	133
20	HPTLC chromatogram of callus extracts of selected plants	134
21	HPTLC analysis on leaf extract of hardened plants of <i>E. glandulosum</i>	135
22	HPTLC chromatogram of leaf extracts of hardened plants of <i>E. glandulosum</i>	136
23	Comparitive quantitative estimation of quercetin content	137

List of Plates

LIST OF PLATES

Plate No	Title
1	Effect of 2,4-D on callus formation in leaf explants
2	Effect of 2,4-D on callus formation in internode and nodal explants
3	Effect of IAA and BA on callus formation in leaf explants
4	Effect of IAA and BA on callus formation in internode explants
5	Effect of IAA and BA on callus formation in nodal explants
6	Propagation of shoots from nodal explants of <i>E.glandulosum</i>
7	Induction of root on <i>in vitro</i> grown plants of <i>E.glandulosum</i>
8	Hardening of <i>in vitro</i> grown plants
9	Establishment of hardened plants in the field