

**CONTENTS**

<b>Chapter No.</b>	<b>Topic No.</b>	<b>Title of Topic</b>	<b>Page No.</b>
	I	List of Figures	I
	II	List of Tables	VI
	III	List of Materials	IX
	IV	List of Instruments/Equipments	X
	V	List of Abbreviations	XI
	VI	Abstract	IVX
<b>Chapter-1: Introduction</b>			
<b>1</b>	1.1	Herbal medicines	1
	1.2	Inflammation	1
	1.3	Types of inflammation	2
	1.3.1	Acute inflammation	2
	1.3.2	Chronic inflammation	3
	1.4	Signs and symptoms of inflammation	4
	1.5	Acute Vs. Chronic inflammation	5
	1.6	Inflammatory cascade	6
	1.7	Chemical Anti-Inflammatory Drugs	10
	1.7.1	Steroidal Anti-Inflammatory Drugs (SAIDs)	10
	1.7.2	Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)	10
	1.8	Importance of Herbal medicines	11
	1.9	Limitations of herbal medicine	11
1.10	References	12	
<b>Chapter – 2: Literature review</b>			
<b>2</b>	2.1	Selection of herbs for polyherbal formulation	15
	2.2	Plant profile	19
	2.2.1	<i>Calotropis procera</i>	19
	2.2.1.1	Geographical distribution and ecology	19
	2.2.1.2	Common names	19
	2.2.1.3	Taxonomic classification	20

	2.2.1.4	Morphology	20
	2.2.1.5	Description	21
	2.2.1.6	Chemical constituents	21
	2.2.1.7	Pharmacological effects	22
	2.2.2	<i>Rosa indica</i>	24
	2.2.2.1	Common Names	24
	2.2.2.2	Taxonomic classification	25
	2.2.2.3	Morphology	25
	2.2.2.4	Chemical Constituents	25
	2.2.2.5	Traditional uses of Rose	25
	2.2.2.6	Pharmacological Activities	26
	2.2.2.7	Uses of Rose water	26
	2.2.3	<i>Adhatoda vasica</i>	27
	2.2.3.1	Common names	27
	2.2.3.2	Taxonomic Classification	27
	2.2.3.3	Morphology	28
	2.2.3.4	Chemical constituents	28
	2.2.3.5	Pharmacological activities	29
	2.2.3.6	Traditional Uses	31
	2.3	References	32
<b>Chapter – 3: Aim and Objectives</b>			
<b>3</b>	3.1	Aim	47
	3.2	Objectives	47
	3.3	References	48
<b>Chapter – 4: Preparation of Extracts, Qualitative and Quantitative Analysis</b>			
<b>4</b>	4.1	Introduction	49
	4.1.1	Standardization of the powdered materials as per the WHO guidelines	49
	4.1.1.1	Various Ash Values	49
	4.1.2	Extraction	49
	4.1.2.1	Methods of extraction	50

4.1.2.2	Extraction procedures	50
4.1.3	Qualitative and Quantitative analysis	52
4.1.3.1	Qualitative analysis	52
4.1.3.2	Quantitative analysis	54
4.1.3.3	Introduction of High-performance thin layer chromatography (HPTLC)	57
4.1.3.4	Validation process	64
4.2	Materials and methods	68
4.2.1	Collection and authentication of Plant materials	68
4.2.2	Preparation of powdered material	69
4.2.3	Standardization of the powdered materials as per the WHO guidelines	69
4.2.4	Preparation of Extracts	69
4.2.5	Qualitative Analysis of extracts	73
4.2.6	Quantitative Analysis by High Performance Thin Layer Chromatography	74
4.2.6.1	Method-1 Method development and validation for Gallic acid in <i>Calotropis procera</i> extract by HPTLC	74
4.2.6.2	Method-2 Method development and validation for Rutin in <i>Rosa indica</i> extract by HPTLC	76
4.2.6.3	Method-3 Method development and validation for Vasicine in <i>Adhatoda vasica</i> extract by HPTLC	78
4.3	Results and Discussion	81
4.3.1	Results of Standardization of plant powder	81
4.3.2	Results of Qualitative Phytochemical Screening of extracts	81
4.3.3	Results of Quantitative analysis by HPTLC method	82
4.3.3.1	Quantification of Gallic acid in <i>Calotropis procera</i> extract	82
4.3.3.2	Quantification of Rutin in <i>Rosa indica</i> extract	87
4.3.3.3	Quantification of Vasicine in <i>Adhatoda vasica</i> extract	92

	4.4	Conclusion	98
	4.5	References	99
<b>Chapter – 5: <i>In-silico</i> studies of extracts</b>			
<b>5</b>	5.1	Introduction to <i>In-silico</i> Studies	101
	5.1.1	Molecular docking	103
	5.1.2	Different Softwares used in molecular docking	104
	5.1.3	<i>In-silico</i> studies for inflammatory conditions	106
	5.1.4	Binding energy	108
	5.1.5	Rationale for the Selection of Receptors in Molecular Docking Studies for Anti-Inflammatory Activity	108
	5.1.5.1	TNF- $\alpha$	108
	5.1.5.2	IL-6	110
	5.1.5.3	COX-1 and COX-2	111
	5.2	Methodology	114
	5.2.1	Retrival of phytoconstituents of all three plants	114
	5.2.2	Preparation of Ligands	114
	5.2.3	Retrieval of Target Sequence	115
	5.2.4	Molecular docking with software	116
	5.3	Results and Discussion	116
	5.3.1	Result analysis and Complex interaction visualization	116
	5.3.2	Docking studies	116
	5.3.2.1	3LN1 (COX-2)	117
	5.3.2.2	1ALU (Interleukin 6)	120
	5.3.2.3	3N8Y (COX-1)	124
	5.3.2.4	1TNF (TNF- $\alpha$ )	127
	5.4	Conclusion of <i>In-silico</i> studies	131
	5.5	References	131
<b>Chapter – 6: <i>In-vitro</i> studies of extracts</b>			
<b>6</b>	6.1	Introduction of <i>In-vitro</i> studies	134
	6.1.1	Cell-viability assay (MTT Assay)	134
	6.1.1.1	Principle of MTT Assay	135

	6.1.2	Introduction of Cytokines	135
	6.1.2.1	History of cytokine	135
	6.1.2.2	Role of Cytokines	136
	6.1.2.3	Cytokine Detection Methods	138
	6.2	Materials and methods	141
	6.2.1	Cell culture	141
	6.2.2	Cell viability assay by MTT assay	141
	6.2.3	Evaluation of anti-inflammatory activity of extracts by ELISA Test	141
	6.3	Results and Discussion	144
	6.3.1	Cell viability assay	144
	6.3.2	Conclusion of MTT assay	146
	6.3.3	Conclusion of Evaluation of anti-inflammatory activity of extracts by ELISA Test	146
	6.3.3.1	Discussion on IL-4 assay	148
	6.3.3.2	Discussion on IL-10 assay	150
	6.3.3.3	Discussion on TNF- $\alpha$ Assay	152
	6.3.3.4	Discussion on IL-6 Assay	154
	6.4	Conclusion of in-vitro assay	154
	6.5	References	155
<b>Chapter – 7: Formulation development and evaluation</b>			
<b>7</b>	7.1	Introduction	157
	7.1.1	Herbal Formulation	157
	7.1.2	Historical Context	157
	7.1.3	Modern Relevance	157
	7.1.4	Advantages of Herbal Formulations	157
	7.1.5	Challenges and Considerations	158
	7.1.6	Components of drug development	158
	7.1.7	Physiology of the skin	160
	7.1.8	Functions of skin	161
	7.1.9	Advantages of Local drug administration	161

7.1.10	Disadvantages of Local drug administration	162
7.1.11	Limitations of Local drug administration	162
7.1.12	Introduction to Polyherbal Gel	163
7.1.13	Introduction to Polyherbal Spray	165
7.1.14	Optimization by Experimental Design	167
7.1.15	Box-Behnken designs (BBD)	168
7.2	Materials and Methods	169
7.2.1	Development of Polyherbal formulations	169
7.2.1.1	Preparation of Polyherbal gel	169
7.2.1.2	Preparation of Optimised batch of Polyherbal gel	172
7.2.2	Evaluation of Optimised Batch of Polyherbal gel	172
7.2.3	Preparation of Optimized Polyherbal Spray	173
7.2.4	Evaluation parameters of the developed polyherbal Spray	173
7.2.4.1	Assay of developed Polyherbal Gel and Polyherbal Spray	174
7.3	Results and discussion	178
7.3.1	Formulation optimization by Box-Behnken Design	178
7.3.2	Effect analysis of critical variables on responses	179
7.3.2.1	Influence of investigated parameters on Viscosity	179
7.3.2.2	Influence of investigated parameters on Spreadability	185
7.3.2.3	Influence of investigated parameters on pH	191
7.3.3	Establishment of Design Space	197
7.3.3.1	Overlay Plot for predicted design space	197
7.3.4	Evaluation of optimised batch of Polyherbal gel	199
7.3.5	Evaluation of Polyherbal Spray	200
7.3.6	Assay of developed Polyherbal Gel and Polyherbal Spray	202
7.4	Conclusion	208
7.5	References	210

<b>Chapter – 8: <i>In-vivo</i> studies</b>			
<b>8</b>	8.1	Introduction	212
	8.1.1	Systematic Approach in Use of Different Animal Models for Evaluations	212
	8.1.2	Animal Models and Mechanisms for Screening of Anti-Inflammatory Activity	213
	8.1.3	Carrageenan-Induced Paw Edema	214
	8.2	Materials and methods	215
	8.2.1	Skin irritation study	215
	8.2.2	Evaluation of anti-inflammatory activity	216
	8.3	Results and discussion	218
	8.3.1	Skin irritation test	218
	8.3.2	Evaluation of anti-inflammatory activity	219
	8.3.3	Conclusion of in-vivo studies	219
	8.4	References	220
	<b>9.</b>	<b>9.</b>	Summary and Conclusion
Presentations and Publications			225
<b>Annexure-I</b>		Course work Certificate	
<b>Annexure -II</b>		Authentication Certificate of Plants	