# CONTENTS

<table>
<thead>
<tr>
<th>Chapter 1.</th>
<th>Introduction, Review of Literature and Objectives</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td>01</td>
</tr>
<tr>
<td>1.1.1</td>
<td>Ayurveda: Herbal Medicine</td>
<td>03</td>
</tr>
<tr>
<td>1.1.1.1</td>
<td>Advantages of Herbal Medicines</td>
<td>06</td>
</tr>
<tr>
<td>1.1.1.2</td>
<td>Limitations of Herbal Medicines</td>
<td>06</td>
</tr>
<tr>
<td>1.1.1.2.1</td>
<td>Ineffective in Acute Medical Care</td>
<td>06</td>
</tr>
<tr>
<td>1.1.1.2.2</td>
<td>Inadequate Standardization and Lack of Quality Specifications</td>
<td>07</td>
</tr>
<tr>
<td>1.1.1.2.3</td>
<td>Lack of Scientific Data</td>
<td>07</td>
</tr>
<tr>
<td>1.2</td>
<td>Validation and Standardization of Herbal Drug</td>
<td>08</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Validation of Herbal Drug</td>
<td>08</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Need of Validation</td>
<td>08</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Standardization of Herbal Drug</td>
<td>09</td>
</tr>
<tr>
<td>1.2.4</td>
<td>Need of Standardization</td>
<td>09</td>
</tr>
<tr>
<td>1.2.5</td>
<td>Current Regulations for Standardization of Crude Drugs</td>
<td>12</td>
</tr>
<tr>
<td>1.2.6</td>
<td>Quality Control of Herbal Crude Drugs</td>
<td>13</td>
</tr>
<tr>
<td>1.2.6.1</td>
<td>Physical Evaluation</td>
<td>14</td>
</tr>
<tr>
<td>1.2.6.1.1</td>
<td>Cyclic Voltammeter</td>
<td>15</td>
</tr>
</tbody>
</table>
1.2.6.2 Chemical Evaluation 17

1.2.6.2.1 Colorimeter 17

1.2.6.2.2 Spectrophotometer 17

1.2.6.2.3 High Performance Thin Layer Chromatography (HPTLC) 18

1.2.6.2.4 High Pressure Liquid Chromatography (HPLC) and Mass Spectroscopy (MS) 18

1.2.6.3 Biological evaluation 19

1.2.6.3.1 In vivo 20

1.2.6.3.2 In vitro 20

1.2.7 Stability and Shelf-life 20

1.3 Antioxidant, Inflammation and Bio-availability 21

1.3.1 Antioxidants 21

1.3.2 Inflammation 22

1.3.2.1 Anti-inflammatory Action 22

1.3.3 Bio-availability 23

1.4 Review of Literature 24

1.4.1 Withania somnifera: Description 24

1.4.1.1 Physiology 24

1.4.1.2 Soil Conditions for Germination 26

1.4.1.3 Water Management 26
1.4.1.4 Temperature 26
1.4.1.5 Grading of Roots 26
1.4.1.6 Profits from Ashwagandha Cultivation 27
1.4.2 Pharmacological Properties of Ashwagandha 29
1.4.2.1 Antioxidant Effect of Ashwagandha 31
1.4.2.2 Anti-inflammatory Action of Ashwagandha 32
1.4.2.3 Anti-stress Effect of Ashwagandha 34
1.4.2.4 Anti-tumor Properties of Ashwagandha 35
1.4.2.5 Androgenic Activity of Ashwagandha 36
1.4.2.6 Estrogenic Activity of Ashwagandha 37
1.4.2.7 Effects of Ashwagandha on the Alzheimer’s Disease 37
1.4.2.8 Effects of Ashwagandha on the Cardioprotection and Cardiopulmonary System 39
1.4.2.9 Effects of Ashwagandha on the Endocrine System 40
1.4.2.10 Hemopoetic Effect of Ashwagandha 41
1.4.2.11 Hepatoprotective Effect of Ashwagandha 41
1.4.2.12 Hypoglycemic and Diuretic Effect of Ashwagandha 42
1.4.2.13 Immunomodulatory Activities of Ashwagandha 42
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4.2.14</td>
<td>Effects of Ashwagandha on Nervous System</td>
<td>43</td>
</tr>
<tr>
<td>1.4.2.15</td>
<td>Rejuvenating Effect of Ashwagandha</td>
<td>44</td>
</tr>
<tr>
<td>4.3</td>
<td>General Toxicity Studies</td>
<td>45</td>
</tr>
<tr>
<td>1.4.4</td>
<td>Phytochemicals of Ashwagandha</td>
<td>47</td>
</tr>
<tr>
<td>1.4.4.1</td>
<td>Withanolides in Ashwagandha</td>
<td>47</td>
</tr>
<tr>
<td>1.4.4.2</td>
<td>Other Components in Ashwagandha</td>
<td>53</td>
</tr>
<tr>
<td>1.4.5</td>
<td>Biosynthesis Pathway of Withanolides</td>
<td>56</td>
</tr>
<tr>
<td>1.4.6</td>
<td>Pharmacological Activities of Individual Withanolides</td>
<td>59</td>
</tr>
<tr>
<td>1.4.6.1</td>
<td>Withaferine A</td>
<td>59</td>
</tr>
<tr>
<td>1.4.6.2</td>
<td>Withanolide A</td>
<td>60</td>
</tr>
<tr>
<td>1.4.6.3</td>
<td>Withanoside (IV-V)</td>
<td>61</td>
</tr>
<tr>
<td>1.4.6.4</td>
<td>Withanolide B</td>
<td>62</td>
</tr>
<tr>
<td>1.4.6.5</td>
<td>1,2 Deoxywithastramonolide</td>
<td>63</td>
</tr>
<tr>
<td>1.4.6.6</td>
<td>Withanone</td>
<td>63</td>
</tr>
<tr>
<td>1.4.7</td>
<td>A Biotechnological Approach to Withanolide Production</td>
<td>65</td>
</tr>
<tr>
<td>1.4.8</td>
<td>Effects of Environmental Parameters on Active Components</td>
<td>67</td>
</tr>
<tr>
<td>1.4.9</td>
<td>Status of Indian Herbal Medicine in International Market</td>
<td>67</td>
</tr>
</tbody>
</table>
1.5 Aims and Objectives

1.5.1 Scope of Work

1.5.2 Aims and Objectives

1.5.3 Plan of Work for Attaining the Objectives

Chapter 2. Influence of Macronutrient Deficiencies on Molybdenum Content in Ashwagandha Roots and its Correlation with Withanolide Content

2.1 Introduction

2.2 Material and Methods

2.2.1 Chemicals

2.2.2 Plant Materials

2.2.3 Determination of Nitrogen, Phosphorus, Potassium and Calcium

2.2.4 Determination Molybdenum (Mo)

2.2.5 Preparation of Root Extracts for Determination of Withanolides Content

2.2.6 Preparation of Standard Withanolide Solutions

2.2.7 Preparation of Colour Reagent for Total Withanolide Determination

2.2.8 Determination of Total Withanolides (TW) Content
2.2.9 Quantification of Major Withanolides (MW)  
2.2.10 Estimation of free radical scavenging activity by 2, 2-diphenyl-1-picryl-hydrazil (DPPH) method  
2.2.11 Statistical analysis  
2.3 Results  
2.3.1 Symptoms of Nitrogen Deficiency  
2.3.2 Symptoms of Phosphorous Deficiency  
2.3.3 Symptoms of Potassium Deficiency  
2.3.4 Symptoms of Calcium Deficiency  
2.3.5 Major Withanolide (MW) by HPTLC  
2.3.6 Total withanolides (TW) Content by Spectrophotometric Analysis  
2.3.7 Molybdenum Content  
2.3.8 Free Radical Scavenging Activity  
2.4 Discussion  
2.5 Conclusions  

Chapter 3 Characterization of Withanolide Rich Fraction (WRF) by using HPTLC and Thin Layer Chromatography-2, 2-Diphenyl-1-picrylhydrazyl (TLC-DPPH) Method  
3.1 Introduction  
3.2 Materials and Methods
3.2.1 Chemicals
3.2.2 Plant Materials
3.2.3 Preparation of Root Extracts for Determination of Withanolide Content
3.2.3.1 Extraction by Chaurasiya et al. (2008)
3.2.3.2 Isolation of WRF by Chaurasiya et al. (2008) With Some Modification
3.2.4 Preparation of Standard Solutions
3.2.5 Quantification of Withanolides by High Performance Thin Layer Chromatography (HPTLC)
3.2.6 DPPH Test
3.2.7 Image Processing by Means of the ImageJ Program
3.2.8 Limits of Detection and Quantification
3.2.9 Validation of the Method
3.2.9.1 Linearity
3.2.9.2 Selectivity
3.2.9.3 Accuracy
3.2.9.4 Precision
3.2.10 Statistical analysis
3.3 Results
### 3.3.1 Yields of Major Withanolides in Extracts

#### 3.3.2 Antioxidant Potential of Components of WRF by TLC-DPPH Method

### 3.4 Discussion

### 3.5 Conclusions

## Chapter 4 Studies on the stability, Shelf-life and Biological Activity of WRF and its Components

### 4.1 Introduction

### 4.2 Materials and Methods

#### 4.2.1 Chemicals

#### 4.2.2 Plant Material

#### 4.2.3 Preparation of Sample for Stability Study

#### 4.2.4 Estimation of Total Antioxidant Capacity of WRF by Cyclic Voltammeter

#### 4.2.5 Estimation of Shelf-life and Stability of Individual Component of WRF by HPTLC

#### 4.2.6 First Order Kinetics Equation

#### 4.2.7 Estimation of Biological activity by Rat Paw Edema Model

##### 4.2.7.1 Experimental Protocol Approval

##### 4.2.7.2 Animal

##### 4.2.7.3 Acute Oral Toxicity Tests for WRF
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.7.4</td>
<td>Experimental Design</td>
<td>116</td>
</tr>
<tr>
<td>4.2.8</td>
<td>Statistical analysis</td>
<td>117</td>
</tr>
<tr>
<td>4.3</td>
<td>Results</td>
<td>118</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Total Antioxidant Capacity of WRF by Cyclic Voltammeter</td>
<td>118</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Stability of Individual Components of WRF by HPTLC</td>
<td>123</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Biological Activity of WRF by Carrageenan Induced Rat Paw Edema Model</td>
<td>132</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>134</td>
</tr>
<tr>
<td>4.5</td>
<td>Conclusion</td>
<td>136</td>
</tr>
</tbody>
</table>

**Chapter 5**  
**Hepatoprotective Effect of WRF in Acetaminophen Intoxicated Rat**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>139</td>
</tr>
<tr>
<td>5.2</td>
<td>Materials and Methods</td>
<td>141</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Chemicals</td>
<td>141</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Plant Material</td>
<td>141</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Preparation of WRF</td>
<td>141</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Animals</td>
<td>141</td>
</tr>
<tr>
<td>5.2.5</td>
<td>Acute Oral Toxicity Tests for WRF</td>
<td>141</td>
</tr>
<tr>
<td>5.2.6</td>
<td>Experimental Design</td>
<td>142</td>
</tr>
<tr>
<td>5.2.7</td>
<td>Serum Analysis</td>
<td>143</td>
</tr>
</tbody>
</table>
5.2.8 Estimation of Oxidative Stress
5.2.9 Estimation of Total Antioxidant Capacity (TAC)
5.2.10 RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis
5.2.10.1 RNA Extraction
5.2.10.2 RT-PCR Analysis
5.2.11 Histological Examination
5.2.12 Statistical Analysis
5.3 Results
5.3.1 Characterization of WRF
5.3.2 Acute Toxicity Studies
5.3.3 Effects of WRF on Serum Parameters
5.3.4 Effects of WRF on Plasma Lipid Parameters
5.3.5 Effect of WRF on Antioxidant Parameters
5.3.6 Effect of WRF on TNF-α, IL-1β, iNOS and COX-II mRNA Expression
5.3.7 Histopathological Observations
5.4 Discussion
5.5 Conclusions
Chapter 6  Evaluation of the Bioavailability of Major Withanolides of Ashwagandha

6.1  Introduction  168
6.2  Materials and Methods  171
6.2.1  Standard Withanolides  171
6.2.2  HPLC-MS Analysis  171
6.3  Results  175
6.4  Discussion  179
6.5  Conclusions  181

Chapter 7  Summary and Conclusions  182

7.1  Summary  183
7.1.1  Preamble: Integration of Ayurveda with Modern Medicine  183
7.1.2  Ashwagandha: A Chosen Herb for Validation Standardization and Stability Studies  183
7.1.3  Pertinent Issues to Address the Problem  184
7.1.3.1  Quality Control in the Farmer’s Field  185
7.1.3.2  Efficient Extraction of Roots  185
7.1.3.3  Fractionation and Quantification of Withanolides by HPTLC  185
7.1.3.4  Simple Quick Antioxidant Assay of Withanolides  185
7.1.3.5 Quicker Physical Technique (Cyclic Voltammeter) for Quality Assessment at Different Stages in the Production of Formulation and for Shelf life

7.1.3.6 Biological Assessment of Anti-inflammatory and Hepatoprotective Activities

7.1.3.7 Bioavailability

7.1.4 Conclusions

7.2 Accomplishments

7.3 Limitations of the Work

7.4 Future Prospects

Bibliography

List of Publications

Awards and Certificates

Errata