## CHAPTER

Chapter I	Introduction	1 - 52	
1.1 Research Background			
1.2 Phytochemical character	isation of <i>Taverniera cuneifolia</i>		
1.2.1 Lipids			
1.2.2 Carbohydrates			
1.3 Importance of plant seco	ndary metabolites		
1.3.1 Phenolics	· · ·		
1.3.2 Phenolic Acids			
1.3.3 Flavonoids			
1.3.4 Stilbenes			
1.3.5 Lignans			
1.3.6 Tannins			
1.3.7 Alkaloids			
1.3.8 Saponins			
1.3.9 Terpenoids			
1.4 Characterisation of phyto	ocompounds		
1.4.1 Maceration			
1.4.2 Percolation			
1.4.3 Infusion			
1.4.4 Digestion			
1.4.5 Sonication/ Ultrasonic	Extraction		
1.4.6 Microwave Assisted Ex	xtraction (MAE)		
1.4.7 Marinated Extraction	· · · · ·		
1.4.8 Reflux/ Hydro-Distilla	tion Extraction		
1.4.9 Soxhlet Extraction			
1.4.10 Steam distillation			
1.4.11 Ultra-High Pressure Extraction (UHPE)			
1.4.12 Accelerated Solvent Extraction (ASE)			
1.4.13 Solid Phase Extraction (SPE)			
1.4.14 Supercritical Fluid Extraction (SFE)			
1.5 Chromatography			
1.5.1 High Performance Thin	n Layer Chromatography (HPTLC)		
1.5.2 High-Performance Liquid Chromatography (HPLC)			
1.5.3 Liquid Chromatography and Mass Spectroscopy (LCMS)			
<b>1.6</b> Analytical Method Validation			
1.6.1 Parameters for Method Validation			
1.6.2 Specificity study			
1.6.3 Linearity and Range Study			
1.6.4 Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) Study			
1.6.5 Precision Study	1.6.5 Precision Study		

1.6.6 Accuracy Study		
1.6.7 Robustness Study		
1.6.8 Solution Stability Study		
1.6.9 System Suitability Study		
1.6.10 Stability Indicating Assay Method		
1.7 National and International Scenario of Herbal Market		
1.7.1 National and International Status of Licorice		
1.8 Hypothesis		
1.9 Objectives		

\_

Chapter II	<b>Review of Literature</b>	53-84			
2.1 Distribution and Ecology					
2.2 Phlyogenetic status					
2.3 Geographical Distribution					
2.4 Botanical Description of Tavern	2.4 Botanical Description of <i>Taverniera</i> genus:				
2.4.1 Ecology					
2.4.2 Pharmacognostic study					
2.4.3 Ethnobotanical uses					
2.5 Phytochemistry, Pharmacologica	al and Bioactive compounds				
2.5.1 Primary metabolites	2.5.1 Primary metabolites				
2.5.2 Secondary metabolites					
2.5.3 Phenolic compounds					
2.5.4 Terpenoids					
2.5.5 Sterols					
2.6 Pharmacological activities	2.6 Pharmacological activities				
2.6.1 Cytotoxic activity	2.6.1 Cytotoxic activity				
2.6.2 Anticancer activity	2.6.2 Anticancer activity				
2.6.3 Anti-HIV activity					
2.6.4 Anti-inflammatory activity					
2.6.5 Antimicrobial activity					
2.6.6 Analgesic and antipyretic					
2.6.7 Antioxidant activity					
2.6.8 Antitussive activity					
2.6.9 Gastroprotective activity					
2.6.10 Antiulcerative activity					
2.6.11 Memory enhancing activity					
2.6.12 Nematicidal activity					
2.6.13 Spasmolytic action					
2.6.14 Sedative activity					
2.6.15 Wound healing activity					
2.7 General uses					

<ul> <li>3.1. Selection and segregation of appropriate germplasm from different bio-geographical zones of Gujarat.</li> <li>3.1.1 Electrical conductivity of soil</li> <li>3.1.2 Apparatus</li> <li>3.2 Standardization and validation of sugars (sweeteners) and amino acids</li> <li>3.2.1 Preparation of Plant Extracts</li> <li>3.2.2 Phytochemical analysis</li> <li>3.2.3 Chromatographic fingerprinting of plant powder</li> <li>3.2.4 Preparation of sample</li> <li>3.2.5 Chromatographic conditions for HPTLC</li> <li>3.2.6 Standardisation and validation of Sugars:</li> <li>3.2.7 Reagents and chemicals</li> <li>3.2.8 Standard and reagent preparation</li> <li>3.2.9 Preparation of Plant extract solution</li> <li>3.2.10 Sample application</li> <li>3.2.11 Sample development</li> <li>3.2.13 Specificity</li> <li>3.2.13.2 University (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Accuracy as recovery</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17.1 Sample development</li> <li>3.2.18 Application</li> <li>3.2.19 Regenta and chemicals</li> <li>3.2.19 Regenta and chemicals</li> <li>3.2.13.2 Precision</li> <li>3.2.13.2 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.17.1 Sample development</li> <li>3.2.17.1 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.3.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of Acity phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of Acity phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of LC Samples</li> <li>3.3.2.2 Preparation of LC Samples</li> </ul>	Chapter III	Materials and Methods	85 - 105	
3.1.1 Electrical conductivity of soil         3.1.2 Apparatus         3.2 Standardization and validation of sugars (sweeteners) and amino acids         3.2.1 Preparation of Plant Extracts         3.2.2 Phytochemical analysis         3.2.3 Chromatographic fingerprinting of plant powder         3.2.4 Preparation of sample         3.2.5 Chromatographic conditions for HPTLC         3.2.6 Standardisation and validation of Sugars:         3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Method validation         3.2.13 Specificity         3.2.13 Apprecision         3.2.13 Apprecision         3.2.13 Apprecision         3.2.13 Apprecision         3.2.13 Apprecision         3.2.13 Apprecision         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.18 Standardisation and validation of Amino acids         3.2.19 Transple application         3.2.17 Sample Preparation         3.2.17 Sample development	3.1. Selection and segregation of ap	ppropriate germplasm from different bio-geo	ographical	
3.1.2 Apparatus         3.2 Standardization and validation of sugars (sweeteners) and amino acids         3.2.1 Preparation of Plant Extracts         3.2.2 Phytochemical analysis         3.2.3 Chromatographic fingerprinting of plant powder         3.2.4 Preparation of sample         3.2.5 Chromatographic conditions for HPTLC         3.2.6 Standardisation and validation of Sugars:         3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17 Sample development	zones of Gujarat.			
3.2 Standardization and validation of sugars (sweeteners) and amino acids         3.2.1 Preparation of Plant Extracts         3.2.2 Phytochemical analysis         3.2.3 Chromatographic fingerprinting of plant powder         3.2.4 Preparation of sample         3.2.5 Chromatographic conditions for HPTLC         3.2.6 Standardisation and validation of Sugars:         3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Method validation         3.2.13.2 Linearity         3.2.13 A Precision         3.2.13 A Precision         3.2.13 A Precision         3.2.13 A Precision         3.2.13 Repeatability (system precision)         3.2.13 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17 Sample development         3.2.17 Sample development         3.2.17 Sample development         3.2.17 Sample development         3.2.17 Sample development     <	3.1.1 Electrical conductivity of soil	l		
<ul> <li>3.2.1 Preparation of Plant Extracts</li> <li>3.2.2 Phytochemical analysis</li> <li>3.2.3 Chromatographic fingerprinting of plant powder</li> <li>3.2.4 Preparation of sample</li> <li>3.2.5 Chromatographic conditions for HPTLC</li> <li>3.2.6 Standardisation and validation of Sugars:</li> <li>3.2.7 Reagents and chemicals</li> <li>3.2.8 Standard and reagent preparation</li> <li>3.2.9 Preparation of Plant extract solution</li> <li>3.2.10 Sample application</li> <li>3.2.11 Sample development</li> <li>3.2.13 Method validation</li> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.4 Precision</li> <li>3.2.13.4 Precision</li> <li>3.2.13.4 Precision</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standardisation and validation of Amino acids</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.2.18.4 Standardisation and validation of Amino acids</li> <li>3.2.19 Reagents and chemicals</li> <li>3.2.19 Reagents and chemicals</li> <li>3.2.11.5 Reagents and chemicals</li> <li>3.2.12 Freagents and chemicals</li> <li>3.2.13 Sample development</li> <li>3.2.13.7 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.18 Th Reagents, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.1.2 Apparatus			
3.2.2 Phytochemical analysis         3.2.3 Chromatographic fingerprinting of plant powder         3.2.4 Preparation of sample         3.2.5 Chromatographic conditions for HPTLC         3.2.6 Standardisation and validation of Sugars:         3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.14 Standardisation and validation of Amino acids         3.2.17 Robustness         3.2.18 Standard and reagent preparation         3.2.17 Robustness         3.2.18 Standard and reagent preparation         3.2.17 Robustness         3.2.17 Robustness         3.2.17 Sample Preparation         3.2.17.1 Sample application         3.2.17.2 Sample development         3.2.17.3 Sample development         3.2.17.3 Sample development         3.2.17.3 Sample development         3.2.17.1 Sample application         3.2.17.2 Sample development         3.2.17.3 Sample devisitation <td>3.2 Standardization and validation</td> <td>of sugars (sweeteners) and amino acids</td> <td></td>	3.2 Standardization and validation	of sugars (sweeteners) and amino acids		
3.2.3 Chromatographic fingerprinting of plant powder         3.2.4 Preparation of sample         3.2.5 Chromatographic conditions for HPTLC         3.2.6 Standardisation and validation of Sugars:         3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13.1 Specificity         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17 Sample Preparation         3.2.17 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample development         3.2.17.4 Sample development         3.2.17.5 Accuracy as recovery         3.2.18 Hethod validation of active phyto-constituents of <i>T. cuneifolia</i> 3.2.17.1 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample devization <td>3.2.1 Preparation of Plant Extracts</td> <td></td> <td></td>	3.2.1 Preparation of Plant Extracts			
<ul> <li>3.2.4 Preparation of sample</li> <li>3.2.5 Chromatographic conditions for HPTLC</li> <li>3.2.6 Standardisation and validation of Sugars:</li> <li>3.2.7 Reagents and chemicals</li> <li>3.2.8 Standard and reagent preparation</li> <li>3.2.9 Preparation of Plant extract solution</li> <li>3.2.10 Sample application</li> <li>3.2.11 Sample development</li> <li>3.2.12 Plate derivatisation</li> <li>3.2.13 Method validation</li> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.14 Precision</li> <li>3.2.15 Accuracy as recovery</li> <li>3.2.16 Repeatability (system precision)</li> <li>3.2.17 Robustness</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17 Sample development</li> <li>3.2.17 Sample development</li> <li>3.2.17.1 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.3.17 Rubustness</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.3.1.7 Rubustness</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.3.17 Rubustness</li> <li>3.3.17 Rubustness</li> <li>3.3.12 TLC analysis</li> <li>3.3.2.12 C-MS/MS analysis</li> <li>3.3.2.14 Preparation of LC samples</li> </ul>	3.2.2 Phytochemical analysis			
<ul> <li>3.2.5 Chromatographic conditions for HPTLC</li> <li>3.2.6 Standardisation and validation of Sugars:</li> <li>3.2.7 Reagents and chemicals</li> <li>3.2.8 Standard and reagent preparation</li> <li>3.2.9 Preparation of Plant extract solution</li> <li>3.2.10 Sample application</li> <li>3.2.11 Sample development</li> <li>3.2.12 Plate derivatisation</li> <li>3.2.13 Method validation</li> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample application</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.2.17.1 Sample application</li> <li>3.1.17.2 Sample development</li> <li>3.1.18 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of CL samples</li> </ul>	3.2.3 Chromatographic fingerprinti	ing of plant powder		
<ul> <li>3.2.6 Standardisation and validation of Sugars:</li> <li>3.2.7 Reagents and chemicals</li> <li>3.2.8 Standard and reagent preparation</li> <li>3.2.9 Preparation of Plant extract solution</li> <li>3.2.10 Sample application</li> <li>3.2.11 Sample development</li> <li>3.2.12 Plate derivatisation</li> <li>3.2.13 Method validation</li> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.18 Method validation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.1.18 Reagents and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.11 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of CL samples</li> </ul>				
3.2.7 Reagents and chemicals         3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Robustness         3.2.18 Method validation of Amino acids         3.2.17 I Sample Preparation         3.2.17 Sample development         3.2.17 Sample development         3.2.17.1 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample derivatisation         3.3.18 Method validation         3.3.18 Method validation         3.3.11 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2.1 Preparation of LC samples <td>3.2.5 Chromatographic conditions</td> <td>for HPTLC</td> <td></td>	3.2.5 Chromatographic conditions	for HPTLC		
3.2.8 Standard and reagent preparation         3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17 Sample Preparation         3.2.17.1 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample derivatisation         3.2.17.3 Sample derivatisation         3.2.18 Method validation         3.3.11 Reagents, standards, and solutions         3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis	3.2.6 Standardisation and validation of Sugars:			
3.2.9 Preparation of Plant extract solution         3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Linearity         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17.1 Sample Preparation         3.2.17.2 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample derivatisation         3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i> 3.3.1 Standardization and validation of Glycyrrhizin         3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples	3.2.7 Reagents and chemicals			
3.2.10 Sample application         3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Method validation         3.2.13 Specificity         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17.1 Sample Preparation         3.2.17.2 Sample development         3.2.17.2 Sample development         3.2.17.3 Sample derivatisation         3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i> 3.3.1 Standardization and validation of Glycyrrhizin         3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples	3.2.8 Standard and reagent preparat	tion		
3.2.11 Sample development         3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17.1 Sample Preparation         3.2.17.2 Sample development         3.2.17.3 Sample development         3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i> 3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples	3.2.9 Preparation of Plant extract se	olution		
3.2.12 Plate derivatisation         3.2.13 Method validation         3.2.13 Specificity         3.2.13.1 Specificity         3.2.13.2 Linearity         3.2.13.3 Sensitivity (limits of detection and limit of quantification)         3.2.13.4 Precision         3.2.13.5 Accuracy as recovery         3.2.13.6 Repeatability (system precision)         3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17.1 Sample application         3.2.17.2 Sample development         3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i> 3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples	3.2.10 Sample application			
<ul> <li>3.2.13 Method validation</li> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2.1C-MS/MS analysis</li> <li>3.2.1 Preparation of LC samples</li> </ul>	3.2.11 Sample development			
<ul> <li>3.2.13.1 Specificity</li> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17.1 Sample Preparation</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample development</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.2.1 Preparation of LC samples</li> </ul>	3.2.12 Plate derivatisation			
<ul> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17.1 Sample Preparation</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.13 Method validation			
<ul> <li>3.2.13.2 Linearity</li> <li>3.2.13.3 Sensitivity (limits of detection and limit of quantification)</li> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17.1 Sample Preparation</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.13.1 Specificity			
<ul> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.2.12 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>				
<ul> <li>3.2.13.4 Precision</li> <li>3.2.13.5 Accuracy as recovery</li> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.2.12 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.13.3 Sensitivity (limits of detec	tion and limit of quantification)		
<ul> <li>3.2.13.6 Repeatability (system precision)</li> <li>3.2.13.7 Robustness</li> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.13.4 Precision			
3.2.13.7 Robustness         3.2.14 Standardisation and validation of Amino acids         3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17.1 Sample application         3.2.17.2 Sample development         3.2.18 Method validation         3.2.18 Method validation         3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.2.12 TLC analysis         3.3.2.1 Preparation of LC samples	3.2.13.5 Accuracy as recovery			
<ul> <li>3.2.14 Standardisation and validation of Amino acids</li> <li>3.2.15 Reagents and chemicals</li> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3 Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validations</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.13.6 Repeatability (system prec	cision)		
3.2.15 Reagents and chemicals         3.2.16 Standard and reagent preparation         3.2.17 Sample Preparation         3.2.17.1 Sample application         3.2.17.2 Sample development         3.2.17.3 Sample derivatisation         3.2.18 Method validation         3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i> 3.3.1 Standardization and validation of Glycyrrhizin         3.3.1.1 Reagents, standards, and solutions         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples	3.2.13.7 Robustness			
<ul> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3 Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.14 Standardisation and validation	on of Amino acids		
<ul> <li>3.2.16 Standard and reagent preparation</li> <li>3.2.17 Sample Preparation</li> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3 Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.15 Reagents and chemicals			
<ul> <li>3.2.17.1 Sample application</li> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.2.18 Method validation</li> <li>3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>		ation		
<ul> <li>3.2.17.2 Sample development</li> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.17 Sample Preparation			
<ul> <li>3.2.17.3 Sample derivatisation</li> <li>3.2.18 Method validation</li> <li>3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.17.1 Sample application			
<ul> <li>3.2.18 Method validation</li> <li>3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.17.2 Sample development			
<ul> <li>3.3: Standardization and validation of active phyto-constituents of <i>T. cuneifolia</i></li> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.17.3 Sample derivatisation			
<ul> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.2.18 Method validation			
<ul> <li>3.3.1 Standardization and validation of Glycyrrhizin</li> <li>3.3.1.1 Reagents, standards, and solutions</li> <li>3.3.1.2 TLC analysis</li> <li>3.3.2 LC-MS/MS analysis</li> <li>3.3.2.1 Preparation of LC samples</li> </ul>	3.3: Standardization and validation	of active phyto-constituents of T. cuneifolia	а	
3.3.1.1 Reagents, standards, and solutions         3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples				
3.3.1.2 TLC analysis         3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples				
3.3.2 LC-MS/MS analysis         3.3.2.1 Preparation of LC samples				
3.3.2.1 Preparation of LC samples	•			
	· · · · · · · · · · · · · · · · · · ·	on for LC-MS/MS		

3.3.2.3 Liquid chromatography-mass spectrometry condition

3.3.2.4 Method Validation

3.3.2.4.1 Linearity

3.3.2.4.2 Specificity

3.3.2.4.3 Accuracy as Recovery

3.3.2.4.4 Precision

3.3.2.4.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

3.3.3 Simultaneous method development and validation of phytoconstituents using HPLC

3.3.3.1 System suitability

3.3.3.2 Linearity, LOD and LOQ

3.3.3.3 Accuracy as recovery

3.3.3.4 Precision

3.3.3.5 Robustness

3.3.4 Assay of liquiritigenin, apigenin, genistein, glabridin, glycyrrhizin, stigmasterol and  $\beta$ -sitosterol from plant extracts:

3.3.6 LC-MS/MS-Q-TOF analysis of Targeted metabolites

3.3.6.1 Sample Preparation

3.3.6.2 LC-MS/MS-Q-TOF instrument parameters

3.4: Purification of the active fractions by Column chromatography.

3.4.1 Extraction of sample for column chromatography:

3.4.2 Column Chromatography

3.4.3 LC-MS-Q-ToF Analysis for untargeted plant metabolite

3.4.3.1 LC-MS/MS-Q-TOF analysis of Targeted metabolites from column chromatography

3.4.3.2 Sample Preparation

3.4.3.3 LC-MS/MS-Q-TOF instrument parameters

3.4.3.4 GCMS Analysis

3.4.3.4.1 GCMS Analysis of Column fraction

3.4.3.4.2 GCMS Analysis of T. cuneifolia leaves and seed extract

3.4.3.5 Acquisition Parameters

Chapter IVResults and Discussion106 - 2114.1: Selection and segregation of appropriate germplasm from different biogeographical<br/>zones of Gujarat4.1.1 Description and classification

- 4.1.1 Description and classification
- 4.1.2 Distribution
- 4.1.3 Observations
- 4.1.3.1 Morphology
- 4.1.3.2 Ecology
- 4.1.3.3 Climatic parameters
- 4.1.3.4 Soil Chemical characters
- 4.1.4 Discussion

4.2: Standardization and validation of sugars (sweeteners) and amino acids.

4.2.1 Solvent Extractive value

4.2.2 Fingerprinting analysis

4.2.3 Standardisation and validation of Sugars

4.2.4 Chromatographic results

4.2.4.1 Method validation

4.2.5 Discussion

4.2.6 Standardisation and validation of Amino acids

4.2.7 Chromatographic results

4.2.8 Method validation

4.2.9 Discussion on amino acids

4.3: Standardization and validation of active phyto-constituents of T. cuneifolia.

4.3.1 Standardisation and validation of Glycyrrhizin using LC-MS/MS

4.3.2 TLC of *T. cuneifolia* and *G. glabra* 

4.3.3 LC-MS/MS summary

4.3.4 Method validation summary

4.3.4.1 Linearity

4.3.4.2 Specificity

4.3.4.3 Accuracy as Recovery

4.3.4.4 Precision

4.3.4.5 Limit of Detection (LOD) and Limits of Quantitation (LOQ)

4.3.5 Glycyrrhizin quantification in plant extracts using LC-MS/MS

4.3.6 Simultaneous method development of phytoconstituents using HPLC

4.3.7 Validation result

4.3.7.1 Specificity and selectivity

4.3.7.2 System suitability

4.3.7.3 Sensitivity

4.3.7.4 Linearity

4.3.7.5 Accuracy as recovery

4.3.7.6 Precision

4.3.7.7 Robustness

4.3.8 Assay of liquiritigenin, apigenin, genistein, glabridin, glycyrrhizin and stigmasterol and  $\beta$ -Sitosterol from plant extracts

4.3.9 LC-MS/MS-Q-TOF analysis of Targeted metabolites

4.3.10 Discussion

4.4: Purification of the active fractions by Column chromatography Column chromatography

4.4.1 GC-MS Analysis result

4.4.1.1 GC Analysis root (Nonpolar fraction)

4.4.2 LC-MS-Q-ToF For Metabolomics study of *T. cuneifolia* 

4.4.2.1 LC-MS/MS-Q-ToF analysis of Targeted metabolites

4.4.2.2 LCMS-QTOF For Untargeted Metabolomics study of T. cuneifolia root

4.4.2.3 Primary metabolites detected in LCMS-Q-ToF

4.4.2.4 Secondary metabolites		
4.4.2.4.1 Phenolic compounds		
4.4.2.4.2 Terpenoidal glycosides		
4.4.2.4.3 Alkaloids		
4.4.2.4.4. Terpenes		
4.4.2.4.5 Sterols		
4.5 Conclusion		
4.6 Suggestions		
Chapter VI	References	212 - 255
Publication		