

Contents

| | |
|-----------------------------|-------|
| Acknowledgement | xxiv |
| List of Figures | xxxii |
| List of Tables | xxxiv |
| List of Abbreviations | xxxvi |

Chapter 1

| | |
|-------------------------------------------------------|----------|
| Introduction..... | 1 |
| 1.1 About the plant <i>Rheum</i> | 4 |
| 1.2 Origin and distribution of species | 5 |
| 1.3 Potential Benefits | 7 |
| 1.4 Ethnobotanical Information | 8 |
| 1.5 <i>Rheum</i> : Nutraceutical uses | 9 |
| 1.6 Phytochemistry | 9 |
| 1.7 Methods for assessment of Genetic Diversity | 11 |
| 1.7.1 Biochemical Markers..... | 12 |
| 1.8 Research Hypothesis | 13 |

Chapter 2

| | |
|----------------------------------------------------------|-----------|
| Review of Literature | 15 |
| 2.1 Experimental Plant | 15 |
| 2.2 Origin and Distribution of species | 17 |
| 2.3 Morphological Characteristics | 18 |
| 2.4 Major <i>Rheum</i> species | 18 |
| 2.5 Pharmacological actions and Potential Benefits | 21 |
| 2.6 Phytochemistry | 22 |
| 2.6.1 Anthraquinones and anthrones | 22 |
| 2.6.2 Stilbenes | 24 |
| 2.6.3 Flavonoids | 26 |
| 2.6.4 Essential oils | 27 |
| 2.6.5 Sterols and Polyphenols | 27 |

| | |
|-----------------------------------------------------------------------|----|
| 2.7 Pharmacological Studies | 31 |
| 2.8 Genetic Diversity Assessment | 36 |
| 2.9 Methods of Genetic Diversity Analysis | 38 |
| 2.9.1 Morphological markers | 39 |
| 2.9.2 Cytological markers | 39 |
| 2.9.3 Biochemical markers | 39 |
| 2.9.4 Molecular markers | 40 |
| 2.9.4.1 Non-PCR-Based Techniques | 41 |
| 2.9.4.2 Markers Based on Amplification Techniques (PCR-Derived) | 41 |
| 2.9.4.3 DNA sequence-based techniques | 45 |
| 2.10 Characterization of Genetic Diversity using RAPD markers | 45 |
| 2.11 Characterization of Genetic Diversity using ISSR markers | 47 |
| 2.12 Threat Status and Conservational measures | 49 |

Chapter 3

| | |
|----------------------------------------------------------------|-----------|
| Materials and Methods..... | 51 |
| 3.1 Materials | 51 |
| 3.1.1 Selection of Sampling sites | 51 |
| 3.1.2 Criteria for Sampling | 51 |
| 3.1.3 Collection of Plant Materials | 52 |
| 3.1.4 Details of Population Size | 53 |
| 3.1.5 Laboratory instruments, Plastic wares, Glass wares | 56 |
| 3.2 Methods | 56 |
| 3.2.1 Sterilization of Glass wares and Plastic wares | 56 |
| 3.2.2 Phytochemical Analysis for active component | 57 |
| 3.2.2.1 Preparation of Plant Extract | 57 |
| 3.2.2.2 Qualitative Phytochemical analysis | 58 |
| 3.2.2.3 Quantitative Phytochemical Analysis | 61 |
| 3.2.3 Antimicrobial Activity of Plant Extracts | 62 |
| 3.2.3.1 Test Organism | 62 |
| 3.2.3.2 Preparation of inoculum | 63 |
| 3.2.3.3 Screening of Antimicrobial assay | 63 |

| | |
|----------------------------------------------------------------------|----|
| 3.2.3.4 Statistical analysis | 64 |
| 3.2.4 Standardization of protocol for Isolation of Genomic DNA | 64 |
| 3.2.4.1 Selection of plant material for DNA Extraction | 64 |
| 3.2.4.2 Genomic DNA Extraction | 64 |
| 3.2.4.2.1 Solutions and Reagents | 64 |
| 3.2.4.2.2 DNA Extraction Protocol | 65 |
| 3.2.4.3 Quantification of Isolated DNA | 68 |
| 3.2.4.4 Qualitative Analysis of Isolated DNA | 69 |
| 3.2.4.5 Dilution Preparation | 69 |
| 3.2.5 Molecular Marker Analysis | 70 |
| 3.2.5.1 Screening of RAPD Primers | 70 |
| 3.2.5.2 Screening of ISSR Primers | 75 |
| 3.2.6 Gel Electrophoresis | 79 |
| 3.2.7 Data Analysis | 81 |

Chapter 4

| | |
|-----------------------------------------------------------------------------|-----------|
| Results | 83 |
| 4.1 Phytochemical Analysis for active components | 83 |
| 4.1.1 Yield Value Determination | 84 |
| 4.1.2 Qualitative Phytochemical Analysis | 85 |
| 4.1.3 Quantitative Phytochemical Analysis | 88 |
| 4.1.4 Antimicrobial Activity Test | 92 |
| 4.2 Standardization of protocol for Isolation of Genomic DNA | 98 |
| 4.2.1 DNA Extraction | 98 |
| 4.2.2 Quantification of Isolated DNA | 98 |
| 4.2.3 Dilution Preparation | 104 |
| 4.3 Molecular Marker Analysis | 104 |
| 4.3.1 Random Amplified Polymorphic DNA (RAPD) Analysis | 104 |
| 4.3.1.1 Assessment of Genetic Diversity among population | 105 |
| 4.3.1.2 RAPD Polymorphism | 107 |
| 4.3.1.3 Genetic Variability and Analysis of Molecular Variance (AMOVA)..... | 110 |
| 4.3.1.4 Genetic Distance | 111 |

| | |
|-----------------------------------------------------------------------|------------|
| 4.3.1.5 Cluster Analysis | 111 |
| 4.3.2 Inter Simple Sequence Repeats (ISSR) Analysis | 114 |
| 4.3.2.1 Genetic Diversity among population | 114 |
| 4.3.2.2 Genetic Variability | 114 |
| 4.3.2.3 ISSR Polymorphism | 116 |
| 4.3.2.4 Genetic Relationship | 119 |
| 4.3.2.5 Analysis of Molecular Variance (AMOVA) | 120 |
| Chapter 5 | |
| Discussion | 122 |
| 5.1 Phytochemical Screening for active components | 122 |
| 5.2 DNA Extraction from Juvenile leaves of <i>Rheum</i> species | 125 |
| 5.3 Random Amplified Polymorphic DNA (RAPD) Analysis | 126 |
| 5.3.1 Genetic Diversity among population | 126 |
| 5.3.2 Population Genetic Differentiation | 127 |
| 5.3.3 RAPD Analysis | 128 |
| 5.3.4 Cluster Analysis | 129 |
| 5.4 Inter Simple Sequence Repeats (ISSR) Analysis | 129 |
| 5.4.1 Genetic Diversity among population | 130 |
| 5.4.2 Genetic Differentiation among population | 131 |
| 5.4.3 ISSR marker efficiency among populations..... | 132 |
| 5.4.4 Relationship among populations | 133 |
| Chapter 6 | |
| Conclusion | 135 |
| Chapter 7 | |
| Summary | 138 |
| References | 144 |