CONTENTS

1. INTRODUCTION 1

2. REVIEW OF LITERATURE 9

2.1 Role of 18S rDNA in Molecular phylogeny 13

2.2 The ITS sequences of the Nuclear Ribosomal DNA 13

2.3 The rbcL Gene and its role in phylogenetic study 14

2.4 The matK Gene as a tool for phylogenetic study 15

2.5 Secondary metabolites and medicinal properties of Solanum 16

3. MATERIALS AND METHODS 18

3.1 Materials 18

3.2 Methods 18

3.2.1 DNA extraction from leaves 18

3.2.2 Target DNA segments and PCR primer designing 25

3.2.3 DNA Amplification reactions 25

3.2.3.1 Amplification of 18S rRNA 27

3.2.3.2 Amplification of ITS 27

3.2.3.3 Amplification of rbcL 29

3.2.3.4 Amplification of matK 29
3.2.4 Nucleotide sequencing

3.2.4.1 Purification of PCR products

3.2.4.2 Cycle sequencing

3.2.4.3 Purification of the cycle sequencing product

3.2.4.4 Loading the samples

3.2.5 Restriction digestion studies

3.2.5.1 Single digestion conditions

3.2.5.2 Double digestion conditions

3.2.6 Agarose gel electrophoresis (AGE)

3.2.7 Sequence alignment

3.2.8 Sequence characteristics

3.2.9 Phylogenetic analyses

3.2.10 Selection of substitution model and Bayesian analyses

3.2.11 Cluster analysis

3.2.12 Total protein profiling

3.2.12.1 Total protein extraction

3.2.12.2 Estimation of total protein

3.2.12.2.1 Preparation of Bradford’s reagent

3.2.12.2.2 Preparation of working solution
3.2.12.2.3 Assay of protein

3.2.12.3 SDS-PAGE

3.2.13. Phytochemical screening

3.2.13.1. Alkaloid test

3.2.13.2 Terpenoid test

3.2.13.3 Flavonoid test

3.2.13.4 Cardiac glycosides test

3.2.13.5 Tannin test

3.2.13.6 Saponin test

3.2.14 Extraction of total alkaloid

3.2.15 Quantification of solasodine

3.2.15.1 Extraction and purification of solasodine

3.2.15.2 Colorimetric determination of solasodine

4. RESULTS AND DISCUSSION

4.1 Collection of leaves

4.2 DNA Extraction

4.3 PCR amplification

4.3.1 Amplification of 18S rRNA

4.3.2. Amplification of rrn ITS
4.3.3 Amplification of \textit{rbcL}.

4.3.4 Amplification of \textit{matK}

4.4 Nucleotide sequence analysis

4.4.1 Nucleotide sequence analysis of \textit{18S rRNA}

4.4.2 Nucleotide sequence analysis of \textit{rrn ITS}

4.4.3 Nucleotide sequence analysis of \textit{rbcL}

4.4.4 Nucleotide sequence analysis of \textit{matK}

4.5 DISCUSSION: Sequence alignment, statistics and comparative utility of the four regions

4.6 Phylogenetic analyses

4.6.1 Phylogenetic analysis of \textit{18S rRNA} gene

4.6.2 Phylogenetic analysis of \textit{rrn ITS}

4.6.3 Phylogenetic analysis of \textit{18S rRNA} and \textit{rrn ITS} in Combination

4.6.4 Phylogenetic analysis of \textit{rbcL}

4.6.5 Phylogenetic analysis of \textit{matK}

4.6.6 Phylogenetic analysis of \textit{rbcL} AND \textit{matK} in Combination

4.6.7 Phylogenetic analysis of both nuclear and chloroplast datasets in combination

4.7 Amplicon Restriction Pattern (ARP)/ PCR- Restriction Fragment
Length Profile (PCR-RFLP)

4.7.1 Amplicon Restriction Patterns with NciI
   4.7.1.1 Restriction digestion analysis of profile PN1 81
   4.7.1.2 Restriction digestion analysis of profile PN2 81
   4.7.1.3 Restriction digestion analysis of profile PN3 89
   4.7.1.4 Restriction digestion analysis of profile PN4 89

4.7.2 Amplicon Restriction Patterns (ARP) with HpaII
   4.7.2.1 Restriction digestion analysis of profile PH1 98
   4.7.2.2 Restriction digestion analysis of profile PH2 98
   4.7.2.3 Restriction digestion analysis of profile PH3 100
   4.7.2.4 Restriction digestion analysis of profile PH4 100
   4.7.2.5 Restriction digestion analysis of profile PH5 101
   4.7.2.6 Restriction digestion analysis of profile PH6 101

4.7.3 Amplicon Restriction Patterns (ARP) with DdeI/ScrFI
   4.7.3.1 Restriction digestion analysis of profile PDS1 103
   4.7.3.2 Restriction digestion analysis of profile PDS2 108

4.7.4 Amplicon Restriction Patterns (ARP) with AvaII
   4.7.4.1 Restriction digestion analysis of profile PA1 109
   4.7.4.2 Restriction digestion analysis of profile PA2 109
4.7.4.3 Restriction digestion analysis of profile PA3 114

4.8 Cluster analysis 114

4.9 Species specific amplicon restriction profile 117

4.10 DISCUSSION 119

4.11 Biochemical analyses 123

4.11.1 Protein Profiling 123

4.11.2 Phytochemical screening 126

4.11.3 Quantification of glycoalkaloid solasodine 129

4.12 DISCUSSION 134

5. CONCLUSION 137

6. REFERENCES 139

7. APPENDICES 161