

Table of Contents

List of Abbreviations	i
List of Figures	iii
List of Tables	vii
Abstract	ix

CHAPTER 1

1.1 Medicinal plants: integration of primordial tradition to contemporary medicines.....	1
1.1.1 India's contribution.....	3
1.2 Quality is the major concern in translation of old wisdom into current prospects.....	4
1.3 Quality parameters of herbal medicines	8
1.3.1 Botanical Identification	10
1.3.2 Physical Assessment.....	10
1.3.3 Chemical Evaluation.....	11
1.3.4 Biological Evaluation	12
1.3.5 Determination of biological and non-biological contaminants	12
1.4 Authentication/identification of botanicals is the first step in quality management	13
1.4.1 Reasons for misidentification and plant derived adulteration	14
1.4.1.1 Careless collection by unskilled collectors.....	14
1.4.1.2 Less knowledge about the authentic source	14
1.4.1.3 Confusion in species identity.....	14
1.4.1.4 Absence of taxonomic key characters during collection	16
1.4.1.5 Deficiency of the authentic species	16
1.4.1.6 Intentional mixing of other vegetative parts of the same plant	16
1.4.1.7 Lack of working taxonomic knowledge	17
1.4.2 Methods for traceability of botanicals	17
1.4.2.1 Morphological Identification.....	18
1.4.2.2 Microscopic Identification.....	19
1.4.2.3 Analytical chemical methods.....	19
1.4.2.4 DNA based methods- from genome profiling to DNA barcoding	20

1.5 The project	29
1.5.1 Scope of the project	29
1.5.2 Hypothesis.....	29
1.5.3 Objectives	30
1.6 Organization of the thesis	30

CHAPTER 2

2.1 Internal transcribed spacers (ITS) and its properties.....	31
2.2 Plant DNA barcoding and Internal transcribed spacers	32
2.3 ITS and traceability of herbals	35
2.3.1 <i>In silico</i> sequence analysis	36
2.3.2 Endpoint and realtime PCR assays	37
2.3.3 High resolution melting curve analysis of DNA barcodes (BAR-HRM).....	38
2.3.4 Barcode based PCR-Restriction fragment length polymorphism (RFLP).....	39
2.3.5 Chip based identification of medicinal plants.....	40
2.3.6 Loop mediated isothermal amplification (LAMP).....	40
2.3.7 Next generation sequencing (NGS)	41
2.4 Description of the plants	42
2.4.1 “Shankpushpi” the controversial identity	42
2.4.1.1 <i>Convolvulus microphyllus</i> Sieb. Ex Spreng.....	43
2.4.1.2 <i>Evolvulus alsinoides</i> (L.) L.....	45
2.4.1.3 Comparative biological activities studies of <i>C. microphyllus</i>	46
and <i>E. alsinoides</i>	
2.4.1.4 Methods for correct identification of Shankpushpi plants.....	47
2.4.2 <i>Terminalia arjuna</i> (Roxb.)Wight & Arn	50
2.4.2.1 Taxonomic status	50
2.4.2.2 Chemical constituents	50
2.4.2.3 Biological activities	50
2.4.2.4 Adulterants.....	53
2.4.2.5 Studies for authentication of <i>T. arjuna</i> bark.....	54

CHAPTER 3

3.1 Materials	57
3.1.1 Instruments and Equipments	57
3.1.2 Plastic wares and glasswares	59
3.1.3 Chemicals and solvents	59
3.1.4 Fine chemicals	59
3.1.5 General reagents and solutions	60
3.1.6 Biological Samples	61
3.1.7 Softwares and <i>in silico</i> tools	61
3.1.8 DNA reference standards.....	62
3.1.9 Commercial kits.....	62
3.2 Experimental methods	63
3.2.1 Collection of plant samples	63
3.2.1.1 <i>Convolvulus microphyllus</i> and <i>Evolvulus alsinoides</i>	63
3.2.1.2 Stem bark of <i>T. arjuna</i> and its adulterant/allied species.....	63
3.2.2 Preliminary identification and validation of the collected plant samples.....	64
3.2.2.1 Morphological analysis.....	64
3.2.2.2 Microscopical Evaluation	64
3.2.3 Genomic DNA isolation, purification and analysis.....	67
3.2.3.1 DNA isolation from leaves of <i>C. microphyllus</i> and <i>E. alsinoides</i>	67
3.2.3.2 DNA isolation from stem bark of <i>Terminalia</i> species.....	68
3.2.4 PCR amplification of ITS locus.....	70
3.2.4.1 Selection of universal primer pairs for ITS region	70
3.2.4.2 Evaluation of PCR enhancers	71
3.2.4.3 Amplification reaction of ITS region	71
3.2.4.4 Amplification of ITS1 and ITS2.....	72
3.2.4.5 Agarose gel analysis for PCR products	72
3.2.5 Bidirectional sequencing of ITS amplicons.....	72
3.2.5.1 Purification of PCR amplicons	73
3.2.5.2 Cycle Sequencing.....	74

3.2.5.3	Quality analysis and editing of sequences	75
3.2.5.4	Assembly of sequences	75
3.2.5.5	Verification and <i>in silico</i> analysis of the sequences using BLAST	75
3.2.5.6	Alignment, sequence characterization and tree based species identification ...	76
3.2.6	Development of species specific primers	76
3.2.7	Development of species specific PCR assays	77
3.2.7.1	Development of assays for <i>C. microphyllus</i> and <i>E. alsinoides</i>	78
3.2.7.2	Development of PCR assays for <i>Terminalia</i> species	80
3.2.8	Agarose gel electrophoresis for PCR products analysis	82

CHAPTER 4

4.1	Collection of plant samples.....	83
4.1.1	<i>C. microphyllus</i> and <i>E. alsinoides</i>	83
4.1.2	<i>T. arjuna</i> and its adulterant/allied species.....	85
4.2	Macroscopic and microscopic verification of collected plant material	87
4.2.1	<i>C. microphyllus</i> and <i>E. alsinoides</i>	87
4.2.2	<i>Terminalia</i> species	91
4.3	Genomic DNA isolation, purification and analysis	99
4.3.1	DNA isolation from leaves of <i>C. microphyllus</i> and <i>E. alsinoides</i>	99
4.3.2	DNA isolation from stem bark of <i>Terminalia</i> species	101
4.4	PCR facilitators and amplification of ITS locus	107
4.4.1	Reaction optimization for ITS locus	107
4.4.2	PCR inhibition and role of PCR additives	108
4.5	Bidirectional sequencing, assembly and characterization of ITS/ ITS1/ITS 2 amplicons	112
4.5.1	Sequencing and quality analysis of reads	112
4.5.2	Sequence assembly and analysis.....	113
4.5.2.1	Sequence assembly and verification	113
4.5.2.2	ITS sequences of Shankpushpi and <i>Terminalia</i> species	115
4.5.2.3	Sequence alignment and characterization.....	118
4.5.2.4	Tree based identification of species	120
4.6	PCR methods for correct species identification.....	122

4.6.1 Primer designing and their <i>in silico</i> analysis	122
4.6.2 Development of PCR methods for correct species identification.....	128
4.6.2.1 Development of PCR assay for Shankpushpi species	131
4.6.2.2 Development of PCR methods for <i>Terminalia</i> species	140
CHAPTER 5	
5.1 Summary and conclusion.....	155
5.2 Limitations and future conducts	157
Appendix	159
References	165
Scientific Contributions	193

