DNA-based Simultaneous Identification of Three *Terminalia* Species Targeting Adulteration

Sonal Sharma¹,², Neeta Shrivastava¹

¹BV Patel Pharmaceutical Education and Research Development Centre; ²Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Submitted: 27-11-2015  Revised: 28-12-2015  Published: 07-07-2016

**ABSTRACT**

**Background:** Various parts of three *Terminalia* species, namely, *Terminalia arjuna* (stem bark), *Terminalia bellirica* (fruit), and *Terminalia chebula* (fruit) are widely known for their therapeutic principles and other commercial values. However, stem bark of *T. bellirica* and *T. chebula* along with *Terminalia tomentosa* are reported as adulterants of *T. arjuna*. Correct botanical identification is very critical for safe and effective herbal drugs. DNA-based identification approaches are advancing the conventional methods and sometime proved more beneficial. **Objective:** The purpose of the study was to develop polymerase chain reaction (PCR) method using internal transcribed spacer (ITS) region to ascertain the identity of *T. arjuna* herbal material as well as detection of mixing of other three *Terminalia* species. **Materials and Methods:** DNA from stem barks samples were isolated and subjected to ITS region amplification and sequencing. Sequences were compared for polymorphic nucleotides determination to develop species-specific primers. Final primers were selected on the basis of *in silico* analysis and experimentally validated. PCR assays for botanical identification of *Terminalia* species were developed. Sensitivity testing and assay validation were also performed. **Results:** The PCR assays developed for *Terminalia* species were resulted in definite amplicons of the corresponding species. No cross-reactivity of the primers was detected. Sensitivity was found enough to amplify as low as 2 ng of DNA. Mixing of DNA in various concentrations for validation also proved the sensitivity of assay to detect original botanicals in the mixture. The developed methods proved very specific and sensitive to authenticate Arjuna bark to develop evidence-based herbal medicines. **Key words:** Botanical identification, internal transcribed spacer, polymerase chain reaction, stem bark, *Terminalia*

**SUMMARY**

- Internal transcribed spacer-based species-specific polymerase chain reaction (PCR) assays were developed to authenticate *Terminalia arjuna* stem bark and to identify substitution/adulteration of *Terminalia bellirica* and *Terminalia chebula* in the genuine starting material.
- Definite amplicons were obtained specific to particular species and the assay was found of profound sensitivity to amplify as low as 2 ng of DNA.
- Results of method validation proved that the assay can identify adulterant *Terminalia* species even when present in lower amounts.
- The DNA barcodes and PCR methods can also be used to identify *Terminalia bellirica* and *T. chebula* related herbal medicinal material.

**INTRODUCTION**

Bark of *Terminalia arjuna* (Roxb) Wight and Arn. and fruits of *Terminalia bellirica* (Gaertn.) Roxb. as well as *Terminalia chebula* Retz., are well known for their therapeutic uses in Ayurveda.¹⁻³ All the three tree species belong to the family Combretaceae. Most prominently, curative effects of *T. arjuna* are clinically proven for cardiovascular and hepatic disorders.¹¹ Triterpenoids present in the stem bark of *T. arjuna* are cardioprotective whereas tannins and flavonoids are responsible for anticancer properties.¹² In addition to therapeutic values, all these tree species are also known for their significant role in timber, tasar silk, dyeing, leather, paper, resin, and soap industries.¹³ However, stem barks of *T. bellirica* and *T. chebula* along with *Terminalia tomentosa* Wight and Arn. have also been reported as potent adulterants of *T. arjuna* bark, in order to attain high demands of arjuna-based drugs in the ayurvedic and herbal market.¹⁶⁻¹⁷ Use of these three *Terminalia* species as the filler material in *T. arjuna* can reduce its curative effects and can pose serious health risk on consumers. Although, few therapeutic activities of the stem bark of these three *Terminalia* species have been reported, but, they are not extensively evaluated for any accountable therapeutic value similar to *T. arjuna*.⁸ In addition, chemical composition of these stem bark different form Arjuna bark, can work synergistically or antagonistically with the bioactive principles of *T. arjuna*. Thus, adulteration and...
mixing of these three species can significantly affect commercialization of T. arjuna-based drugs. Genome-based identification methods either can facilitate the conventional macroscopy, microscopy, and chemical profiling techniques to develop a comprehensive approach for authentication and scientific validation of a species or can work as standalone technique.[18] In addition, DNA-based methods are more stable as seasonal, environmental, and age-related factors do not affect the reliability of results. They are also good to identify processed material in which taxonomic key characters are in reduced form.[19] In the present study, polymerase chain reaction (PCR) methods were developed to ascertain the identity of Arjuna bark raw material and to detect it along with cogenic adulterants, in mixed herbal material. PCR methods were validated for specificity and sensitivity. PCR methods were developed using species-specific polymorphic sites present in the internal transcribed spacer region of plant nuclear genome. This region is highly polymorphic and high copies are present in the nuclear genome, hence has potential to be amplified in degraded biological material also.[3,12,13]

**MATERIALS AND METHODS**

**Plant collection, DNA isolation, amplification, and sequencing of internal transcribed spacer locus**

Stem bark samples of Terminalia species were collected from different locations of India and authenticated by conventional methods with the help of taxonomists. Voucher specimens with their specimen numbers were submitted in herbarium division of our institute. DNA isolated from bark samples was used for amplification of internal transcribed spacer (ITS) locus with the help of universal primers[14] using method previously reported by Sharma et al.[15] The amplified products were sequenced, edited, and assembled with the help of Sequence Analysis version 5.2 (Applied Biosystems, CA, USA) and Codon Code Aligner (Trial version, Codon Code, Dedham, MA, USA). Basic Local Alignment Search Tool (BLAST) was performed for verification of assembled contigs and sequence boundaries were determined comparing the sequences with the database.[16]

**Selection of plant-specific primers**

ITS sequences of all the four species were aligned with Clustal W tool using MEGA 5.1 to determine polymorphic nucleotides which were used further to develop species-specific primers.[17] Each site was checked manually to develop primers. In silico tools, OligoCalc and Primer3 softwares were used to test each polymorphic site for its feasibility to design suitable primers on the basis of optimal length, optimum GC content, melting temperature compatibility, hairpin formation, secondary structure, and species specificity through primer blast.[18,19] Bioinformatically tested primers were validated experimentally. ITS sequence comparison revealed absence of any polymorphic site between T. arjuna and T. tomentosa for suitable primer development of T. tomentosa while many polymorphic sites were present between and among other three Terminalia species. One forward and reverse primer were developed for authentication of T. arjuna, and primers for T. bellirica and T. chebula were also developed aiming the detection of the adulterants [Table 1].

**Polymerase chain reaction assay for authentication of Terminalia arjuna stem bark**

Primer pair TAF and TAR [Table 1] was used to develop Arjuna-specific identification method. Specificity of the primers and the reactions were evaluated by amplifying DNA of other two species with Arjuna-specific primers. Sensitivity of the method was tested using a range of dilutions of DNA from each species (2, 5, 10, 25, 50, 75, and 100 ng). DNA from stem bark samples of each species collected from various locations were isolated and amplified for validation. The reaction was conducted as follows - 1X Taq buffer, 2.50 mM MgCl$_2$, 200 µM dNTP, primers TAF and TAR 20 pm each, Taq Polymerase 1U, bovine serum albumin (BSA) 0.5 µg/µl following the program 94°C (10 min), 30 cycles of 94°C (1 min), 60°C (45 s), 72°C (1 min), and final extension 72°C (5 min).

**Development of multiplex polymerase chain reaction assay targeting adulteration detection in Terminalia arjuna, sensitivity testing, and validation**

The developed species-specific primers were further used to develop a multiplex PCR assay to ensure the presence and absence of T. arjuna and its adulterant species in a mixture. To reduce the complexity, Arjuna-specific reverse primer (TAR) was dropped and ITS 4 was included as the common reverse primer. The experiment of multiplexing was designed with eight sets of reactions having different combination of primers and DNA template [Table 2]. Sensitivity of the developed method was assessed using a battery of DNA concentrations. Subsequently, DNA of three species was mixed in various ratios (total DNA concentration was approximately 50–60 ng) and subjected to amplification with multiplex PCR assay. The reaction was conducted using PCR components at the concentration of 0.75X Taq buffer, 2.50 mM MgCl$_2$, 400 µM dNTP, 20 pm TAF [Table 1], 1.5 pm TBF [Table 1], 10 pm TCF [Table 1], 15 pm ITS 4, 2U Taq Polymerase, 0.5 µg/µl BSA, 5% dimethyl sulfoxide (DMSO) following the program 94°C (10 min), 35 cycles of 94°C (1 min), 50°C (45 s), 65°C (1 min), and final extension 65°C (5 min).

**RESULTS AND DISCUSSION**

In the fast-growing market of plant-based medicine, quality and efficacy of herbal drugs are still a big bottle neck.[20] Quality parameters are negatively affected due to incorrect identification of the plant species, adulteration as well as nonscientific method of collection in inapt season. Application of DNA-based species identification method is widely growing which can be used individually or can facilitate the conventional approaches for authenticity detection. Moreover, DNA sequence-based approaches enhance the universality, accuracy, and precision in comparison to random DNA fingerprinting techniques. Instantaneously, sequence-inspired methods as simple sequence repeat, sequence-characterized regions, and DNA barcoding are resulted in better effectiveness to economic ratio.[21] Moreover, with advent of NGS technologies, genome-based identification of plant material has become more rapid and vast.[22]

Table 1: Sequence of the primers developed for molecular identification of Terminalia species

| Species name        | Primer name | Primer sequence (5’-3’)                  | Length (bp) | Tm  | CG%  | Approximate product size |
|---------------------|-------------|-----------------------------------------|-------------|-----|------|-------------------------|
| Terminalia arjuna   | TAF         | GCCAAGGTACTCCAACGGGAGG                 | 20          | 62  | 60   | 269 bp with TAR and 517 bp with ITS 4 |
|                     | TAR         | GAGGCTTTTGGAGGCACGAT                 | 20          | 55  | 60   | 150 bp                  |
| Terminalia bellirica| TBF150      | GTGATCTAAGCCCCAGCAG                    | 20          | 58  | 60   | 150 bp                  |
| Terminalia chebula  | TCF244      | GATGGGAGGAGTGGTCGGGA                  | 20          | 55  | 65   | 244 bp                  |

ITS: Internal transcribed spacer
To develop ITS-based identification method of *T. arjuna* and its adulterant, stem bark samples of all the four species were collected from various parts of the country [Table 3]. ITS region was amplified in all the species after DNA isolation from the collected bark samples. The amplified ITS regions resulted in approximately 700 bp amplicon which was subsequently sequenced. Assembly of raw reads of sequence produced contigs of 667 bp (GeneBank: KF925432.1), 681 bp (GeneBank: KT187391), 720 bp (GeneBank: KC602394.1), and 701 bp (GenBank: KC750922.1) for *T. arjuna* (Pune, vouchered BVPPERD/PP/0811/12), *T. tomentosa* (Udaipur, vouchered BVPPERD/PP/0113/02), *T. bellirica* (Anand, vouchered BVPPERD/PP/0511/13), and *T. chebula* (Anand, vouchered BVPPERD/PP/0511/14), respectively. BLAST analyses of the contigs proved authenticity of the assembled sequences.

Selection of plant-specific primers, development of polymerase chain reaction assay for *Terminalia arjuna* identification

Polymorphic sites were identified in aligned sequence [with Clustal W tool] which was further used to develop species-specific primers for each species [Table 1]. Sequence comparison of *Terminalia* species revealed high homology between ITS sequences of *T. arjuna* and *T. tomentosa*, hence specific primer differentiating these two species could not be developed using ITS sequence. Investigation of sequence information for other loci such as rbcL, psbA-trnH, and matK revealed that only matK region has polymorphic nucleotides between these two *Terminalia* species. The focus of our study is the Arjuna stem bark and being part of chloroplast region, there are less chances of amplification of plastid regions in stem bark samples, therefore PCR method was developed for *T. arjuna*, *T. bellirica*, and *T. chebula*. At first stage, a singleplex method was developed to ascertain the identity of *T. arjuna*. The results showed the specificity of primers of *T. arjuna*, as other than *T. arjuna* and *T. tomentosa*, none of the *Terminalia* species

**Table 2:** Summary of multiplex method development, validation, and results

| Reaction number | DNA added | Amplicons of corresponding species (bp) |
|-----------------|-----------|------------------------------------------|
|                 |           | TA (517 bp) | TB (150 bp) | TC (244 bp) |
| 1               | TA        | +           | +           | +           |
| 2               | TB        | +           | +           | +           |
| 3               | TC        | +           | +           | +           |
| 4               | TA + TB   | +           | +           | +           |
| 5               | TB + TC   | +           | +           | +           |
| 6               | TA + TC   | +           | +           | +           |
| 7               | TA + TB + TC | +     | +           | +           |
| 8               | NTC       | +           | +           | +           |

**Method development**

| Ratio of DNA of TA:TB:TC | Amplicons of corresponding species |
|-------------------------|-----------------------------------|
|                         | TA (517 bp) | TB (150 bp) | TC (244 bp) |
| 1:1:1                   | +          | +           | +           |
| 2:1:1                   | +          | +           | +           |
| 1:2:1                   | +          | +           | +           |
| 1:1:2                   | +          | +           | +           |
| 4:1:1                   | +          | +           | +           |
| 1:4:1                   | +          | +           | +           |
| 1:1:4                   | +          | +           | +           |
| 8:1:1                   | +          | +           | +           |
| 1:8:1                   | +          | +           | +           |
| 1:1:8                   | +          | +           | +           |

**Method validation**

TA: *Terminalia arjuna*; TB: *Terminalia bellirica*; TC: *Terminalia chebula*

NTC: No template control; “+” and “−”: Positive PCR results or no any amplification, respectively; PCR: Polymerase chain reaction

**Table 3:** Locations of plant sample collection

| Species name        | Locations                          |
|---------------------|------------------------------------|
| *Terminalia arjuna* | Ahmedabad, Anand, Dang, DMAPR*, Gandhinagar, Junagarh, Nasik, Pune, Rajpipla, Udaipur |
| *Terminalia tomentosa* | Juagarh, Rajpipla, Udaipur          |
| *Terminalia bellirica* | Ahmedabad, Anand, Gandhinagar, Pune, Rajpipla, Udaipur |
| *Terminalia chebula* | Anand, Gandhinagar, Pune, Rajpipla, Udaipur |

*Directorate of medicinal and aromatic plant research, Anand

**Figure 2:** (a) Two percent agarose gel is showing results of multiplex polymerase chain reaction. Lane 2, Lane 3, and Lane 4 specific to *Terminalia arjuna*, *Terminalia bellirica*, and *Terminalia chebula*, respectively, in control reactions having all the three primers but DNA of only one species. Lanes 5, 6, and 7 are showing polymerase chain reaction results of control reactions having all three primers with DNA of two species. In Lane 8, all the three amplicons are found detectable upon mixing of DNA of all the three species. (b) Sensitivity testing of multiplex polymerase chain reaction assay. Lanes 2 to 8 are showing amplification from of each species having DNA concentrations 2, 5, 10, 25, 50, 75, and 100 ng, respectively. Lane 1: No template control; M: GeneRuler 50bp DNA Ladder (Thermo Scientific, Massachusetts, United States)
showed amplification. Specificity of the PCR assay was obtained by decrement in concentration of PCR components, especially primers and simultaneously annealing temperatures were increased with reduction in the annealing time. These approaches helped inhibit cross-amplification of the species and gave specific amplicons of 269 bp for *T. arjuna*. The reactions were found sensitive enough to amplify as low as 2 ng of DNA template. Universality of the primers and consistency of the PCR assays were validated through amplification of DNA from samples collected from various locations [Figure 1].

**Development of multiplex polymerase chain reaction assay, sensitivity testing, and validation**

Further, a multiplex reaction was developed utilizing the species-specific forward primers and a common reverse primer. Primers developed for *T. bellirica* and *T. chebula* were also validated experimentally in singleplex PCR. Arbitrary refractory mutation system technique was used to increase specificity of all these primers and subsequent assays. Development of multiplex PCR for a sample having mixture of DNA from three different species using three different primers was found very challenging. Further, it was checked for cross-reactivity and specificity also. Eight individual reactions were set up for the assay. Each reaction tube contained three forward primers (one each from individual *Terminalia* species) and one reverse primer along with other PCR components. These eight reaction tubes were further added with DNA templates of *Terminalia* species as per the experimental design mentioned in Table 2. Results of multiplex PCR assay [Table 2 and Figure 2] exhibited high specificity as all the eight PCR reactions amplified amplicons of very specific size corresponding with the *Terminalia* species was added. Addition of single species DNA resulted in a single band (517 bp for *T. arjuna*, 150 bp for *T. bellirica* and 244 bp for *T. chebula* in control reaction) while addition of two species combination generated two bands specific to the species added. In the reaction with three DNA templates, all the three bands were observed on the agarose gel. The result of multiplex assay showed the specificity of designed primer toward DNA of specific *Terminalia* species [Figure 2a and Table 2]. Development of this multiplex PCR assay claimed lots of optimization efforts. Reduction in salt concentration in PCR buffer and higher concentration of TAF [Table 1] primer resulted in the appearance of *T. arjuna* band. Primer concentration for *T. bellirica* was needed to be reduced for appearance of band specific to *T. arjuna* and *T. chebula*. Five percent DMSO was added which increased the specificity with more clear bands on the agarose gel [Figure 2]. Some modifications in annealing and extension temperatures (from 72 to 65°C) led to more consistent PCR amplification.

Sensitivity of the multiplex assay was assessed using different concentrations of DNA template (2–100 ng) and found to be sensitive as to as low as 2 ng of DNA template of all the three *Terminalia* species when mixed in equal amount [Figure 2b].

Validation of multiplex PCR assay was done by mixing of DNA of *Terminalia* species in different ratios [Table 2]. Results of validation revealed that sensitivity of the assay to detect any *Terminalia* species was not affected much with changes in DNA amount ratios in DNA mixture containing all the *Terminalia* species.

**CONCLUSION**

Thus, PCR assays developed in this study are useful for identification of *T. arjuna*. Concurrently, detection of *T. bellirica* and *T. chebula* is possible if they are used as fillers in genuine raw material of *T. arjuna* bark. PCR methods developed in this investigation have the potential to identify and discriminate three *Terminalia* species with great specificity and sensitivity which can be further utilized as quality control for herbal drug starting material.

**Acknowledgment**

The authors are thankful to UGC and GSBTM, India, for financial assistance. Authors also wish to thank following personnel and institutes for helping in stem bark collection, i.e. Directorate of Medicinal and Aromatic Plant Research and Dr. Mahesh Patel, AAU, Anand; Jawaharlal Nehru Udhyan, Gandhinagar; Dr. H. L. Dadhik, Junagadh Agriculture University; Government Medicinal Botanical Garden, Rajpilpa; Dr. Arun M. Gavat at National Research Institute of Basic Ayurvedic Sciences, Pune and District Forest Officer of Udaipur in 2011 and their staff, Mr. Diggijay Singh, Mr. Pushpendra Thakkar and Mr. Lalit (Rajasthan) and also to Xcelsius Labs Ltd., technical team, Ahmedabad; Dr. C. G. Joshi and Mr. Parakash P. Patil, AAU, Anand for helping in sequencing.

**Financial support and sponsorship**

The project is financially supported by the UGC, New Delhi, and GSBTM, Gandhinagar.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Maulik SK, Talwar KK. Therapeutic potential of *Terminalia arjuna* in cardiovascular disorders. Am J Cardiovasc Drugs 2012;12:157-63.

2. Javanjal H, Raput MS, Agrawal P, Danve V. Pharmacological evaluation of fruits of *Terminalia bellirica* Roxb for antilipid activity. J Complement Integr Med 2012;9:doi: 10.1510/1593-3840.1566.

3. Bag A, Bhattacharyya SK, Chattopadhyay RR. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. Asian Pac J Trop Biomed 2013;3:244-52.

4. Jain S, Yadav PP, Gill V, Vasudeva N, Singla N. *Terminalia arjuna* a sacred medicinal plant: Phytochemical and pharmacological profile. Phytochem Rev 2009;8:491-602.

5. Sarvat M, Das S, Srivastava PS. Estimation of genetic diversity and evaluation of relatedness through molecular markers among medicinally important trees: *Terminalia arjuna* and *T. chebula* and *T. belerica*. Mol Biol Rep 2011;38:5025-36.

6. Sain YK. Illustrated Manual of Herbal Drugs Used in Ayurveda. New Delhi: CSIR and ICMR; 1996.

7. Anonymous. *Terminalia arjuna* (Roxb) Wight & Arn. In: Gupta AK, Tondon N, Sharma M, editors. Quality Standards of Indian Medicinal Plants. New Delhi: Indian Council of Medical Research; 2005. p. 243-52.

8. Gupta PC. Biological and pharmacological properties of *Terminalia chebula* Retz. (Harrtaki) – An overview. Int J Pharm Pharm Sci 2012;4:62-8.

9. Sucher NJ, Carles MC. Genome-based approaches to the authentication of medicinal plants. Planta Med 2008;74:603-23.

10. Chen S, Pang X, Song J, Shi L, Yao H, Han J, et al. A renaissance in herbal medicine identification: From morphology to DNA. Biotechnol Adv 2014;32:1237-44.

11. Joshi K, Chavan P, Warude D, Patwardhan B. Molecular markers in herbal drug technology. Curr Sci 2004;87:159-65.

12. Hollingsworth PM, Graham SW, Little DP. Choosing and using a plant DNA barcode. PLoS One 2011;6:e19254.

13. Yao H, Song J, Liu C, Luo K, Han J, Li Y, et al. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One 2010;5:pi: E13102.

14. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press Inc.; 1990. p. 315-22.
15. Sharma S, Sharma P, Yadav S, Purohit I, Srivastava A, Varma A, et al. Homogenous PCR of heterogenous DNA from phenolic rich barks of Terminalia species for DNA-based adulteration detection. Proc Natl Acad Sci Biol Sect 2015;doi:10.1007/s40011-015-0576-z.

16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.

17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731-9.

18. Kibbe WA. OligoCalc: An online oligonucleotide properties calculator. Nucleic Acids Res 2007;35:W43-6.

19. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3 – New capabilities and interfaces. Nucleic Acids Res 2012;40:e115.

20. Mishra P, Kumar A, Nagireddy A, Mari DN, Shukla AK, Tiwari R, et al. DNA barcoding: An efficient tool to overcome authentication challenges in the herbal market. Plant Biotechnol J 2016;14:8-21.

21. Galimberti A, De Mattia F, Losa A, Bruni I, Federici S, Casiraghi M, et al. DNA barcoding as a new tool for food traceability. Food Res Int 2013;50:55-63.

22. Cheng X, Su X, Chen X, Zhao H, Bo C, Xu J, et al. Biological ingredient analysis of traditional Chinese medicine preparation based on high-throughput sequencing: The story for Liuwei Dihuang Wan. Sci Rep 2014;4:5147.

23. Liu J, Huang S, Sun M, Liu S, Liu Y, Wang W, et al. An improved allele-specific PCR primer design method for SNP marker analysis and its application. Plant Methods 2012;8:34.

24. Henegariu O, Heerema NA, Dolichy SR, Vance GH, Vogt PH. Multiplex PCR: Critical parameters and step-by-step protocol. Biotechniques 1997;23:504-11.

ABOUT AUTHOR

Dr. Neeta Shrivastava is currently working as an assistant director at the BV Patel Pharmaceutical Education and Research Development Centre, Ahmedabad, India. She has 20 years of research and teaching experience. Her research interest includes plant biotechnology, metabolic engineering, molecular markers, phytopharmacology and phytochemistry. She has completed various projects in collaboration with national and international institutions. She had been actively involved in preparation and compilation of monographs of medicinal plants in “Quality Standards of Indian Medicinal Plants” published by the Indian Council of Medical Research, New Delhi. She has published many research as well as review papers in various national and international scientific journals.