CHAPTER 5

Summary and Conclusion

This chapter covers the entire study, its background, objectives, experiments and results obtained in very summaries form. Conclusions drawn from the results are also mentioned. Future conducts and prospective of the study is also described.

5.1 Summary and conclusion

Plants have been using as the medicinal material since time immemorial. In the modern era also the plants are the sources of various chemical drugs. But to stand equal with the synthetic drugs herbal drugs has to pass through strict regulations before reaches to the market. Lack of proper quality control in herbal drug sector is the major concern to provide healthier versions and safe plant based drugs to the consumers. Application of stringent regulatory norms along with new technologies is priori to transform legacy into well organized, safe and efficacious herbal drugs. Scientifically validate & technologically standardized resources of herbal drugs will increase the trust and global acceptance.

Among the various quality parameters correct botanical identification of the starting material to prepare the finished product is the very first and critical step. This project was designed to address the problem of correct botanical identification and plant derived adulteration in herbal drugs. Various approaches are there for authentication of the plant species in medicinal material. Genome dependent molecular technologies are the most advance and independent approach to ascertain the identity of the botanicals. In addition, DNA based methods can be used in synchrony with the conventional practices. The project is targeted to develop PCR methods to ascertain molecular identity of original species as well as its discrimination with other adulterants/ allied species. In this project two highly significant medicinal plants have been aimed. PCR methods have been developed to discriminate two Shankhpushpi plants *viz C. microphyllus* and *E. alsinoides* from each other. Both the plants have similar vernacular identity. Overlapping vernacular name inhibit the use of the right species. In addition, in this project PCR

methods have been developed for establishment of molecular identity of *T. arjuna* stem bark and its discrimination with other *Terminalia* (*T. tomentosa, T. bellirica, T. chebula*) species. PCR assays have been developed using the species specific polymorphic sites exist in the internal transcribed spacer (ITS). ITS is the gene candidate highly recommended for the plant DNA barcoding. Examples have been cited in which ITS region is used to identify and discriminate the plant species. Plants samples of all the species have been collected from various locations and verified by evaluating their morphological and microscopical characteristics. Isolation of DNA has been done in order to amplify and sequence ITS region. Difficulty has been observed in isolation of good quality DNA and amplification of ITS region from Terminalia species. Addition of bovine serum albumin has successfully relieved the PCR inhibition. Amplifide ITS region has been subjected to sequencing. Raw reads have been assembled and the consensus thus build of each species have been verified using BLAST. BLAST search accurately assigned all the species except *T. arjuna* and *T. tomentosa*. Tree based analysis of the sequences has also been performed which showed ITS sequences are variable enough to discriminate Shankhpushpi species where as some ambiguity has been observed in case of *Terminalia* species. Similar to BLAST output, ITS sequences showed close proximity between *T. arjuna* and *T. tomentosa*. Further, ITS of the original species has been aligned with their corresponding adulterants/ allied species to find variable sites. Polymorphic sites have been identified manually and used to develop primers for PCR methods. Among the several possible species specific primers, final selection has been made by analyzing each variable site with in silico tools. Selected oligos has been taken further for experimental validation Singleplex PCR has been developed to validate the primers suitability in three terms: 1) Amplifiability 2) Cross reactivity and 3) Sensitivity. Various optimizations in the PCR conditions and primer sequences for individual species has led to the PCR methods having no cross reactivity between the allied/ adulterant and the original one with optimum sensitivity. Primers performed well in singleplex PCR has been taken further to develop multiplex PCR to identify the species in mixtures. Final multiplex PCR assays for the Shankhpushpi resulted into the two clearly distinguish amplicon of size 200 bp and 596 bp *for C. microphyllus* and *E. alsinoides* respectively. The methods amplify both the species without any cross reactivity proved by putting the appropriate control. Validation of the methods using

simulated blended material also resulted into the two specific amplicons. Similarly, multiplex method has been developed for the Terminalia species also exhibited high specificity. Amplicons of size 517 bp, 150 bp and 244 bp have been obtained specifically from *T. arjuna*, *T. bellirica* and *T. chebula* respectively. Mixing of two species resulted into two bands of corresponding species and all the three amplicons have been obtained upon mixing of all the three species

The PCR assays developed in this project can be used as rapid quality control tool for correct species identification in order to develop scientifically validated herbal drugs. These DNA based approaches will work as the added tool to develop the comprehensive approach to establish the identity of medicinal material related to Shankhpushpi plants and Arjuna stem bark. It is a step forward to make our country global leader in the herbal drug sector. The methods can be taken further to develop benchtop diagnostic kits for authentication of the species in question.

5.2 Limitations and future conducts

Ethical research will not complete without mentioning the conducts need to be performed in future to overcome the limitations of the project.

1. PCR based methods are very prone to degraded or poor quality DNA. Degraded or sheared DNA is hard to amplify which can be a limitation.
2. PCR methods developed in this project can be validated with the other co-generic species also.
3. Other barcoding regions can be included in analysis to make the identification more precise and accurate.
4. Application of DNA barcodes for species identification can be extend to more advance molecular tools as quantitative PCR, BAR-HRM, Microarray etc.