Chapter 5

Summary and Conclusion

Plants have been used as medicinal resources since ancient times, and they continue to provide important compounds for modern drugs. However, to ensure herbal drugs meet the standards of synthetic drugs, they must adhere to strict regulations before reaching the market. The primary challenge in the herbal drug industry is the lack of comprehensive quality control, which is essential for delivering safe and effective plant-based medicines to consumers. Implementing stringent regulatory standards, combined with advanced technologies, is crucial to modernizing and ensuring the safety and efficacy of herbal drugs. Scientifically validated and technologically standardized herbal products will strengthen trust and promote global acceptance.

5.1 Summary and Conclusion

This research investigated DNA isolation and authentication methods for single or polyformulation that contained three polyphenol-rich fruits used in Ayurvedic medicine: *Terminalia bellirica*, *Terminalia chebula*, and *Phyllanthus emblica*.

Here are the key findings:

- High polyphenol and polysaccharide content in these fruits pose significant challenges for DNA extraction and amplification. CTAB extraction protocol often results in poor-quality DNA, limiting the effectiveness of DNA-based authentication.
- Modifications to the extraction protocol, such as adding polyvinylpyrrolidone and increasing buffer strength, were necessary to improve DNA yield and quality.
- High PVP concentrations (up to 1000 mg) during grinding proved most effective in mitigating DNA browning and enhancing PCR amplification, particularly for TC and PE.
- Sensitivity assays revealed that fruit DNA, especially from TC and PE, required higher concentrations for successful amplification compared to leaf-derived samples. This highlights the challenges posed by the complex chemical composition of these fruits.
- Applicability on the market formulation of Baheda, Harde and Amala showed

promising results for TB but raised concerns about potential adulteration in TC and PE products. This highlights the need for reliable authentication methods in the herbal medicine market.

- Digital PCR (dPCR) proved to be a more sensitive and robust method for authenticating these polyphenol-rich fruits compared to conventional PCR. This is particularly relevant for TC and PE, which often yield low-quality DNA.
- For metabarcoding studies, *ITS2* metabarcode (~300 bp) was developed to address amplification challenges posed by DNA degradation in processed herbal products.
- *ITS2* metabarcode region achieved 88.9% amplification efficiency across 45 plant species. However, some species failed to amplify, likely due to the shorter barcode length.
- Two different types of controls were prepared as follows: Control 1) Genomic DNA from plant leaves from different genera belonging to diverse families has been first isolated and pooled into three different groups compromise of three different groups was prepared, 2) genomic DNA (Isolated from plant leaves) pool from different species of the two genera.
- An optimized data analysis pipeline, including filtering for read length, clustering at 98% similarity, and excluding having clusters <5 reads, improved species resolution and detection accuracy in complex mixtures.
- Mock controls demonstrated the effectiveness of the optimized *ITS2* metabarcode and data analysis pipeline in identifying target species within complex mixtures.
- The first type of mock control tested the primer's ability to distinguish target species in mixtures of varying complexity, with some variation in detection rates. The second type assessed the resolution of metabarcode power for closely related species within the same genera, demonstrating its ability to differentiate between these closely related species.
- Later on, the applicability of species-specific PCR and *ITS2* metabarcode was tested on DNA extracted from both mock controls and six commercial Triphala samples.
- Species-specific PCR and metabarcoding analyses revealed a discrepancy in two of the commercial Triphala samples. Specifically, *Phyllanthus emblica* was

absent in T1 and T2 samples, and non-target species were detected, indicating potential adulteration or contamination.

The findings reveal substantial variability in market formulations, raising concerns over quality control in herbal products. Combining DNA-based authentication with analytical authentication for phytochemical validation creates a robust, comprehensive approach to product verification, supporting consumer trust and regulatory compliance in the herbal industry. The optimized DNA extraction, Species-specific PCR, and metabarcoding protocols established in this study lay the groundwork for standardizing DNA-based quality control in complex herbal products, providing a reliable framework for the global acceptance of Ayurvedic formulations.

5.2 Limitations and Future Perspective

To ensure ethical and comprehensive research, it is crucial to acknowledge the limitations of the project and outline future research directions that address these limitations.

1. PCR-based authentication methods do not provide quantitative data nor detect the presence of active therapeutic bioactive compounds.
2. PCR-based methods may give false negative results with degraded or low-quality DNA in processed herbal products. To overcome this, targeting the chloroplast genome and using SCAR or SNP markers, having less than 100 bp amplicon size, can enhance amplification success.
3. Incorporating multiple metabarcoding approaches, improves the precision and accuracy of species identification, especially for closely related species or complex mixtures.
4. Advanced molecular tools like probe-based digital PCR and Bar-HRM offer enhanced sensitivity and specificity for reliable species discrimination.