Chapter 6
Summary and Conclusion
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Chlorophillum borivilianum and Phyllanthus amarus both are important medicinal herbs and extensively used in Indian Systems of Medicine (ISM). C borivilianum, also known as Safed musli has a worldwide distribution to cure general debility, fatigue, weakness and male sterility. The medicinal value of safed musli is due to the presence of steroidal saponins. Phyllanthus amarus is also known as Bhumi amla and has a long history of folk use in drug industry for the treatment of dropsy, urinogenital problems, dysentery, diabetes, skin ulcer, dyspepsia, fever, asthma, bronchial infections, tumour and Hepatitis B virus. The phytoconstituents present in these herbs are multifunctional and cure numerous human ailments. The synthesis of specific metabolites is likely to have been key for survival and diversification of different plant species. A better understanding of the correlation between genes and the functional phenotype of an organism is the true goal of all functional genomics strategies. The multiple functions of secondary metabolites are common and do not contradict their main role for chemical defense and signaling.

The assessment of genetic diversity is important for improvement and conservation of medicinal plants. On the population level, habitat fragmentation, environmental degradation, and over harvesting impose stresses on native plant populations, resulting in declines in density and abundance, decreased fitness, and increased isolation potentially leading to extinction. For many species, genetic diversity is directly related to population size and levels of genetic diversity may affect individual fitness and potential population persistence. Research suggests that populations of wild-collected herbs are falling below minimum viable sizes and are becoming extinct due to collection pressures. Because the majority of medicinal plant species are not cultivated, most materials for herbal remedies originate in native forests. The survival of plant species under different environmental conditions depends on secondary metabolites. The biochemical phenotype of an organism is the final result of interactions between the genotype and the environment (G/E), but it is also modulated by sub-cellular physiological fluctuations that are part of homeostasis. Most of the secondary metabolite structural diversity is generated by differentially
modifying common backbone structures, with the derived compounds having potentially divergent biological activities.

The conclusions drawn from this study on phytochemical and DNA fingerprints of *P. amarus* and *C. horiviliamum* accessions collected from different geographical regions of India are:

- The AFLP results were very precise with EcoRI/MseI primer combinations.
- Each primer combination gave almost same dendrogram and reproducible results.
- The reproducibility of phytochemical fingerprinting was low in comparison to DNA fingerprinting.
- Three base pair extension in primer combinations for selective amplification showed more specificity.
- The numbers of monomorphic, polymorphic and unique loci were same in local market samples as well as in wild plant accessions of these genera.

**Chlorophytum horiviliamum**

- The average polymorphism in *C. horiviliamum* was found 76.19% and 75% at wavelength 254nm and 430nm, while with AFLP marker the average polymorphism was found 35.35% that was much different at population level.
- Some compounds were dominant in these plant accessions. The reason for this could be less impose of environmental conditions on synthesis of these phytoconstituents. However, the content of these compounds were found to vary in accessions collected from different geographical regions.
- The compound with Rf value of 0.03 at wavelength 430nm was present in all accessions collected from different geographical regions of India.
- The compound with Rf value of 0.94 at 430nm was present in all collected accessions except accession numbers 13, 17, 18, 20 and 21, respectively.
- The Rf values of unique compounds at wavelength 254nm present in the accessions were 0.22, 0.29, 0.50; and at 430nm were 0.37, 0.58, 0.71, 0.75, 0.77 and 0.84, respectively. In AFLP fingerprint however, four Unique bands were found in accession numbers 19, 9; 20 and 4 with three EcoRI/MseI primer-1, primer-2 and primer-3.
The accessions from Sangrur and Meerut showed more genetic diversity in comparison to accessions from Indore and Guna, Dehradun and Jamia Hamdard when AFLP fingerprints were used to assess genetic diversity in these accessions.

The accessions from Lucknow and Sangrur showed more genetic diversity in comparison to accessions from Lucknow and Aligarh when phytochemical fingerprints were used to assess genetic diversity in these accessions.

The high content of sarsapogenin (0.988%) was found in accession collected from Nagaur (Rajasthan).

**Phyllanthus amarus**

- There was much diversity regarding metabolites accumulation in root, stem, leaf and seed at 254nm and 615nm wavelengths.
- The phytochemical diversity among organs at wavelength 254nm and 615nm was found 34% and 27.86%, respectively.
- The phyllanthin compound with Rf value 0.48 at 282nm wavelength was present in all collected accessions. The phyllanthin content was varied from one geographical location to another geographical location. Thus the synthesis of this compound highly influenced by environmental conditions.
- Total number of compounds at wavelength 254nm and 615 nm were found 403 and 457 but types of compounds at these wavelengths were 74 and 93, respectively.
- The polymorphism at 254nm and 615nm was found 73.40% and 75.06% while in AFLP fingerprinting the polymorphism was found 62.52%.
- Thirteen unique bands were found with three primer combinations in AFLP fingerprinting while in different organs of *P. amarus*, the number of unique compounds at 254nm were found 7(root), 4(stem), 2(leaf) and 2(seed) and at 615nm were found 6(root), 5(stem), 4 (leaf) and 3(seed), respectively.

**Root**

- The compounds of Rf values 0.19 and 0.93 were dominant as compared to other detected compounds.
The unique compounds at 254nm with Rf values 0.13, 0.28, 0.29, 0.71, 0.74, 0.78 and 0.85 were present in collected accessions from Gorakhpur, Jammu, Agra, Hyderabad, Dehradun, Udhampur and Kathua (accession nos- F, A, E, J, L, D, B) and the unique compounds at wavelength 615 nm with Rf values 0.02, 0.08, 0.17, 0.54, 0.77 and 0.92 were found in collected accession from Dehradun, Bahu fort, Betul, Agra, Betul and Pantnagar (accession nos- l, C, P, E, P and K), respectively. The maximum phyllanthin content was found in accession of Sagar (0.0194 %) while minimum in accession of Batauti (0.0026%).

Stem

The unique compounds with Rf values 0.04, 0.23, 0.29 and 0.62 at 254nm were found in accessions collected from Betul, Bahu fort, Gorakhpur and Gorakhpur (Rf value different) (P, C, F, F), respectively and the unique compounds with Rf values 0.03, 0.07, 0.40, 0.56 and 0.77 at wavelength 615nm were found in accessions collected from Agra, Dehradun, Udhampur, Jammu and Guna, respectively. The maximum phyllanthin content was found in accession of Sagar (0.2056%) while minimum in accession of Batauti (0.0088%).

Leaf

The numbers of compounds were higher in leaf as compared to other organs like stem, seed and root.

The unique compounds with Rf values 0.17 and 0.24 were found in collected accessions from Jamia Hamdard and Jammu, and unique compounds with Rf values 0.09, 0.30, 0.48 and 0.99 at wavelength 615nm were found in collected accessions from Guna, Kota, Aligarh and Hyderabad (Accession no- Q, H, J, M), respectively.

The maximum phyllanthin content was found in accession of Sagar (0.0613%) while minimum in accession of Aligarh (0.387%).

Seed

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The unique compounds with Rf values 0.03 and 0.52 at 254 nm were found in accessions of Chhindwana and Kota (R and M), and unique compounds with Rf values 0.12, 0.24 and 0.32 at wavelength 615 nm were found in accessions collected from Indore, Udhampur and Batal, respectively.

The accessions collected from Basti and Chhindwana showed more genetic diversity as compared to the accessions collected from Hyderabad and Panipnagar with phytochemical fingerprinting.

The maximum phyllanthin content was found in accession of Sagar (0.0294\%\%) while minimum in accession of Udhampur (0.2467\%\%).

**Samples from local markets**

- The samples of *C. horivillanum* purchased from Delhi, Aligarh and Meerut showed similar AFLP fingerprints as fingerprints generated from accessions collected from Aligarh, Chandanpur sanctuary, Nagpur and Akola. The monomorphic, polymorphic and unique loci were 65, 9 and 4 in local market samples that was similar to the accessions collected from Aligarh, Chandanpur sanctuary, Nagpur and Akola, respectively. The phytochemical fingerprints of samples purchased from Delhi, Meerut and Aligarh showed almost secondary metabolite similarities to Hoshiarpur and Nagaur, Dehradun, Kota and Nagaur, respectively.

- The phytochemical fingerprints of *P. amaranus* purchased from Delhi, Aligarh and Agra showed almost similarities to the accessions collected from Gorakhpur and Agra, Gorakhpur and Kota; and Kota, respectively. However, the AFLP fingerprints of samples purchased from Delhi, Aligarh and Agra had only similarities with AFLP fingerprints in accessions collected from Kathua and Bahu fort, Agra and Aligarh, kota and Aligarh, respectively. The monomorphic, polymorphic and unique loci were 14, 27 and 3, in local market samples that was identical to the accessions collected from different geographical regions (Kathua and Bahu fort, Agra and Aligarh, kota and Aligarh) of India.
In the above study, we have found that AFLP markers are more stable and reproducible than the phytochemical markers and thus, are more likely to provide true measurement of genetic diversity. However, phytochemical markers showed more genetic diversity as compared to AFLP markers. Thus, we conclude that both markers i.e. phytochemical and AFLP should be used in combinations for quality assurance of herbal drugs.